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Sphingomonas pokkalii sp. nov., a novel plant associated rhizobacterium isolated from a saline tolerant pokkali rice and its draft genome analysis

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ABSTRACT

Three strains L3B27^T, 3CNBAF, L1A4 isolated from a brackish cultivated pokkali rice rhizosphere were characterised using a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA and *recA* gene sequences revealed that these strains were highly similar among each other and formed a separate monophyletic cluster within the genus *Sphingomonas* with *Sphingomonas pituitosa* DSM 13101^T, *Sphingomonas azotifigens* DSM 18530^T and *Sphingomonas trueperi* DSM 7225^T as their closest relatives sharing 97.9–98.3% 16S rRNA similarity and 91.3–94.0% *recA* similarity values, respectively. The average nucleotide identity (ANI), average amino acid identity (AAI) and digital DNA–DNA hybridisation (dDDH) values between L3B27^T (representative of the novel strains) and its phylogenetically closest *Sphingomonas* species were well below the established cut-off <94% (ANI/AAI) and <70% (dDDH) for species delineation. Further, the novel strains can be distinguished from its closest relatives based on several phenotypic traits. Thus, based on the polyphasic approach, we describe a novel *Sphingomonas* species for which the name *Sphingomonas pokkalii* sp. nov (type strain L3B27^T = KCTC 42098^T = MCC 3001^T) is proposed. In addition, the novel strains were characterised for their plant associated properties and found to possess several phenotypic traits which probably explain its plant associated lifestyle. This was further confirmed by the presence of several plant associated gene features in the genome of L3B27^T. Also, we could identify gene features which may likely involve in brackish water adaptation. Thus, this study provides first insights into the plant associated lifestyle, genome and taxonomy of a novel brackish adapted plant associated *Sphingomonas*.

Introduction

Plant rhizosphere hosts complex diverse microbes whose functions in relation to plant health and growth is still not fully understood. However, plants often depend on this complex microbiota to acquire important nutritional elements which are inaccessible to the plants. In other cases, rhizosphere associated bacteria provide protections against invading soil borne pathogens, improves plant growth by production of phytohormones and help plants to tolerate abiotic stresses such as drought, salinity and more [4,35]. Thus, understanding and harnessing the untapped potential beneficial functions of the plant microbiota can provide sustainable solutions to increase agricultural crop production [20,40]. To achieve this, improvised and detailed knowledge on the composition of the microbiome of healthy plants is required.

As part of an ongoing research survey on the isolation and characterisation of plant beneficial rhizobacteria from pokkali rice; a unique rice variety traditionally grown in coastal saline affected agricultural fields of southern India (Kerala). We isolated many potential novel rhizobacterial isolates using different designed low nutrient bacteriological media and some were characterised as novel taxon [27–29,41]. Among this pool of rhizobacteria, three

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isolates namely, L3B27^T, 3CNBAF and L1A4 belonging to the genus *Sphingomonas* were chosen for this study. Most species of the genus *Sphingomonas* are well known for their capacity to degrade wide range of industrial pollutants and environmental contaminants [33,45]. However, very little is known about *Sphingomonas*-plant beneficial interactions and their genomes. At the time of writing, the genus *Sphingomonas* contains more than 100 species with validly published names, isolated from various environments including different plant habitats such as phyllosphere, rhizosphere and roots [12,43,56]. But, the novel *Sphingomonas* strains described in this study is the first from a crop plant traditionally cultivated in brackish environments.

In this study, we describe three strains L3B27^T, 3CNBAF and L1A4 using a polyphasic taxonomic approach as recommended [23]. On the basis of genotypic, phenotypic, chemotaxonomic and genome data, it is proposed that these three strains L3B27^T, 3CNBAF and L1A4 represent a novel species of the genus *Sphingomonas* for which the name *Sphingomonas pokkalii* sp.nov is proposed. In addition, the novel strains were characterised for their plant associated properties through *in vitro* plate assays. Also, the draft genome was performed to provide first insights into the plant associated lifestyle and brackish adaptation of this interesting novel *Sphingomonas* strain L3B27^T.

Materials and methods

The source of isolation and bacterial media used to obtain the novel strains L3B27^T, 3CNBAF and L1A4 were listed in Table S1. A routine serial dilution procedure was followed to isolate these novel strains [27]. For routine analysis, the strains were subcultured in R2A agar medium and stored at 4 °C. Otherwise, the strains were stored frozen at −80 °C in 10% glycerol stocks as cell suspensions. The reference type strains; *Sphingomonas pituitosa* DSM 13101^T, *Sphingomonas azotifigens* DSM 18530^T, *Sphingomonas trueperi* DSM 7225^T and *Sphingomonas kyeonggiensis* JCM 18825^T were used for comparative taxonomic characterisation.

The genomic DNA was isolated using QIAamp DNA mini kit (Qiagen) according to manufacturer's instructions. The PCR amplification of 16S rRNA and *rec*A genes, sequencing and phylogenetic analysis using different tree construction algorithms were carried out as described [28]. The whole genomic DNA fingerprinting was performed as described before [27] using $(GTG)_5$ primer.

The morphological and physiological properties such as catalase, oxidase, growth in different bacteriological medium, growth at different temperature, pH and NaCl, anaerobic growth, citrate utilisation, indole production, nitrate reduction, methyl red and Voges Proskauer (MR-VP) tests, hydrolysis of starch, pectin, carboxymethyl cellulose, xylan, esculin, urea, tributyrin, DNA, arginine dihydrolase, lysine decarboxylase and antibiotic sensitivity tests were carried out as described by Krishnan et al. [28]. The ability to grow in different natural seawater concentrations (50 and 100%) was checked in R2A broth for 7 days at 30 °C. Acid production from carbohydrates was assessed using the HiMedia HiCarbohydrate kit, according to the manufacturer's instructions, except that the kits were inoculated with culture suspensions made in 0.85% NaCl (w/v), and incubated at 30 °C for up to 2 days.

The cellular fatty acids analysis were carried out as described before [28] except the cells were grown on R2A agar medium for 2 days at 30 °C before harvesting for cellular fatty acids. Importantly, the cells of identical physiological stages were considered for fatty acid analysis. For polar lipid, quinone and polyamine analysis, the cells were grown in PYE broth (0.3% bacteriological peptone, 0.3% yeast extract, pH 7.2). Quinones and polar lipids were extracted from stationary phase grown cells and analysed according to Krishnamurthi et al. [26]. The choline containing lipids were detected using Dragendorff's reagent [13]. The presence of polyamines in L3B27^T was analysed directly without any derivatisation procedures through Liquid chromatography-mass spectrometry (LC–MS). The closest phylogenetic neighbour *S. pituitosa* DSM 13101^T was used as the positive control. Briefly, the lyophilised bacterial biomass of L3B27^T and *S. pituitosa* DSM 13101^T (both 5 mg) was dissolved in 1 ml deionised water and filtered through 0.22 μm N_{66} Posidyne positively charged zeta membrane (Pall Life Sciences), followed by injecting the samples to LC–MS 8045 (Shimadzu, Japan). 70% of solvent A (methanol w/0.1% formic acid) and 30% of solvent B (water w/0.1% formic acid) was used as a mobile phase for the LC analysis on a C18 column followed by mass analysis of the polyamine compounds using electrospray ionisation (ESI)-MS in the scanning mode. 1 mg/ml solution of Sym-homospermidine and spermidine were used as standards. The predominant polyamine was determined based on the comparative peak intensities observed in the LC–MS spectra [5,34].

High quality genomic DNA of L3B27^T was extracted using QiaAmp mini DNA isolation kit as per manufacturer's instructions. The L3B27^T genome was sequenced using Illumina HiSeq 4000 platform with paired ends of 101 base pairs read length and more than 80% of the reads were of high quality with phred score > = 30 (extracted using NGSQC toolkit) [39]. Assembly of the high quality reads was performed using Velvet v.1.2.10 [55]. The scaffolds of the assembled reads were constructed using SSPACE v.3.0 [6]. Denovo genome validation and quality control for the final scaffolds was performed using Bowtie2 v.2.2.2 [31].

Gene prediction and functional annotation was carried out using Rapid Annotation Subsystem Technology (RAST) server [2]. tRNA and rRNA genes were identified using the Aragorn and RNAmmer software programs, respectively [30,32]. The CAZyme categories were predicated using dbCAN database [54]. The identification and classification of the transporters were performed using TransAAP tool based on TransportDB [15]. Circular map for the genome of type strain L3B27^T was carried out using DNAplotter [9]. The calculation of the average nucleotide identity (ANI) was performed using ANIb facility in JSpecies WS [42]. The average amino acid identity (AAI) was determined by using AAI calculator available online in Kostas lab (http://enve-omics.ce.gatech.edu/). *In silico* DNA–DNA hybridisation (DDH) values were calculated using Genome to Genome Distance Calculator (GGDC 2.5) [1].

Plant beneficial traits such as ability to grown in nitrogen free semisolid medium, 1-amino cyclopropane1-carboxylic acid (ACC) deaminase activity, detection of *nifH* gene by PCR amplification and biofilm formation was determined by following the methods of Krishnan et al. [27]. The siderophore production was studied using universal Chrome Azurol S (CAS) agar plate assay as described by Schwyn and Neilands [44] except nutrient broth was used as the basal medium to which the indicator dye CAS was added. These agar plates (CAS + nutrient broth) were inoculated with bacteria and incubated for a week at 30 °C. Formation of yellow halo zone around the bacterial colonies as visualised by change of colour from green to yellow indicates positive siderophore production. The indole-3-acetic acid (IAA) production was checked in Burk's nitrogen free broth supplemented with 0.2% tryptophan and incubated for 3 days at 30 °C followed by IAA quantification as determined by Salkowski assay [18]. The ability to utilise various plant-derived compounds as sole carbon or nitrogen sources was carried out as described earlier [28,41] except the tests were performed by using 24 well microtiter plates and the results were recorded after 7 days at 30 °C.

All the gene sequences generated in this study were submitted to GenBank with the following accessions, 16S rRNA gene; MH251637 to MH251639, *recA* gene; MH294511 to MH294513 and MH294515 to MH294518. The draft genome sequence of L3B27^T was deposited in NCBI with accession number QENQ00000000.

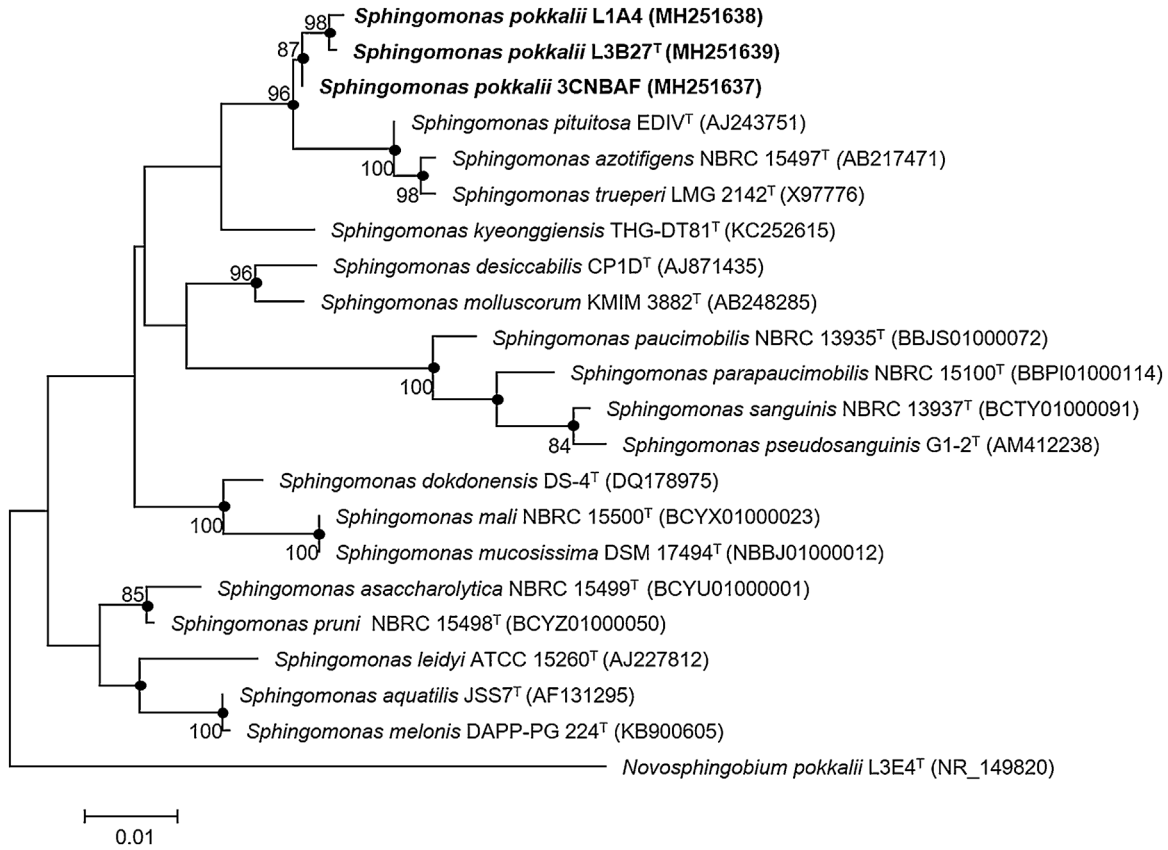

Fig. 1. Maximum likelihood tree constructed using 16S rRNA nucleotide sequences displaying the phylogenetic position of the three novel *Sphingomonas* strains (L3B27^T, L1A4, and 3CNBAF) within the *Sphingomonas* genus. The percentages shown at each branching point represent bootstrap values derived from 1000 replications and values more than 60% are indicated. Bar, 0.01 represents positions per nucleotide position. The outgroup used for this analysis was *Novosphingobium pokkalii* L3E4^T. Dark circles at each nodes shows similar grouping seen in other tree constructing methods (neighbour joining and maximum parsimony).

Results and discussion

S. pokkalii *sp. nov., using polyphasic taxonomy approach*

The 16S rRNA sequence analysis clearly supported the affiliation of the three novel strains L3B27^T, 3CNBAF and L1A4 to the genus *Sphingomonas* (Fig. 1). Pairwise 16S rRNA sequence similarity analysis revealed that the novel strains shared higher 16S rRNA sequence similarities among each other (99.6–99.8%) and exhibited highest sequence similarities to *S. pituitosa* EDIV^T (98.2–98.6%), *S. trueperi* LMG 2142^T (97.9–98.3%) and *S. azotifigens* NBRC 15497^T (97.9–98.3%). The 16S rRNA sequence similarities between the novel strains and with other type strains of the genus *Sphingomonas* was 98.2% or less. In all our 16S rRNA phylogenetic tree construction methods, the novel strains were placed in a separate monophyletic cluster comprising all the other species of the genus *Sphingomonas* (Fig. 1). This cluster was well supported by a higher bootstrap value of 96%. The levels of 16S rRNA sequence similarity among the novel strains and with its nearest *Sphingomonas* type strains were higher than 98.3%. Therefore, we sequenced *recA* gene which was previously used to resolve the sphingomonads group [10,28]. The *recA* gene sequence analysis revealed that the strains L3B27^T, 3CNBAF and L1A4 had higher gene sequence similarity values (99.1–99.3%) among each other indicating that they all belong to a single species. When considering the observations that strains which have *recA* gene similarities higher than 95% mostly shares >70% DNA–DNA hybridisation value at the DNA–DNA relatedness level [17,49]. However, we found that the novel strains shared 94% or less sequence similarity with its closest *Sphingomonas* type strains (Table 1), suggesting that they could represent as a novel

Table 1
Pairwise *recA* gene sequence similarities between the three novel *Sphingomonas* strains and its closest type strain of *Sphingomonas* species.

Strains	Sequence similarity (%)					
	1	2	3	4	5	6
1. L3B27^T	100					
2. L1A4	99.3	100				
3. 3CNBAF	99.1	99.3	100			
4. *S. pituitosa* DSM 13101^T	92.5	92.3	93.0	100		
5. *S. azotifigens* DSM 18530^T	93.0	92.7	93.4	94.7	100	
6. *S. trueperi* DSM 7225^T	93.6	93.4	94.0	94.2	96.1	100
7. *S. kyeonggiensis* JCM 18825^T	91.3	91.1	91.7	93.8	93.4	93.4

Sphingomonas species. Since, *recA* sequence similarities between *S. pituitosa* DSM 13101^T and *S. azotifigens* DSM 18530^T and between *S. azotifigens* DSM 18530^T and *S. trueperi* DSM 7225^T were found to be more than 94% (Table 1). Further, phylogenetic analysis based on partial *recA* gene sequences clearly placed the novel *Sphingomonas* strains as a distinct monophyletic cluster within the genus *Sphingomonas*, irrespective of the tree construction methods used (Fig. 2), which is in agreement with the results of 16S rRNA tree analysis (Fig. 1).

Considering the results from the phylogenetic analysis of two genes, we selected L3B27^T as the representative strain for whole genome sequence analysis to further resolve the taxonomic position of the novel strains. The calculated ANI, AAI and dDDH values between the genomes of L3B27^T and its closest *Sphingomonas* type strains were shown in Table 2. These values are well below the commonly accepted ANI/AAI/dDDH cut-off values proposed for species

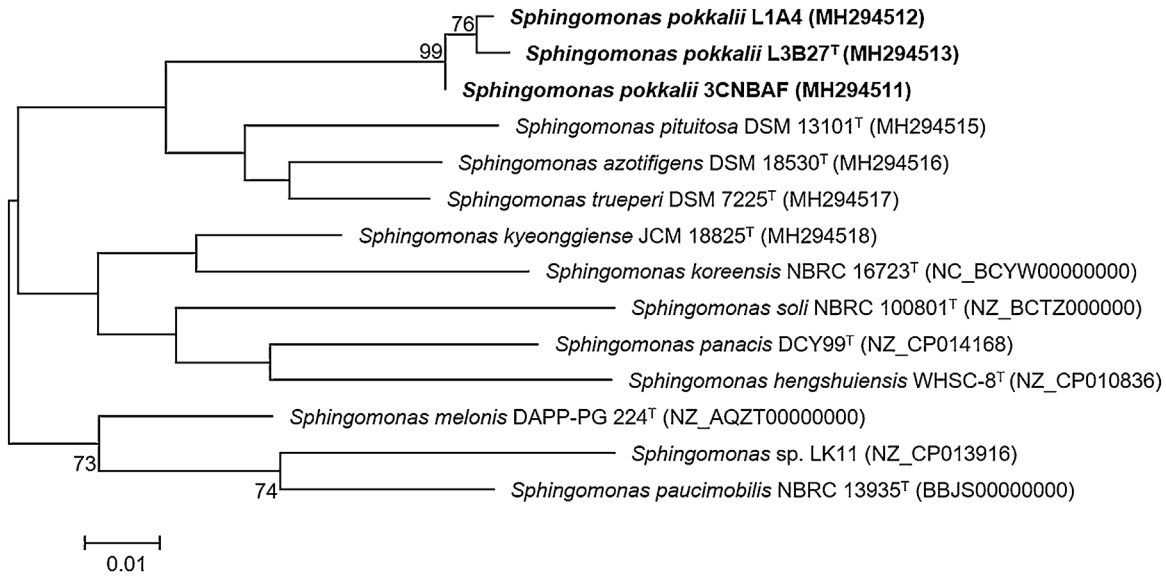

Fig. 2. Maximum likelihood tree constructed using *recA* nucleotide sequences displaying the phylogenetic position of the three novel *Sphingomonas* strains (L3B27^T, L1A4, and 3CNBAF) within the *Sphingomonas* genus. The percentages shown at each branching point represent bootstrap values derived from 1000 replications and values more than 60% are indicated. Bar, 0.01 represents positions per nucleotide position.

Table 2
Genome sequence similarity calculations between strain L3B27^T and its closest *Sphingomonas* type strains.

Strains	ANIb (%)	AAI (%)	dDDH (%)	Tetra z-score
S. pituitosa NBRC 102491^T	83.5	83.6	35.9	0.98737
S. azotifigens NBRC 15497^T	83.9	84.5	36.3	0.98684
S. kyeonggiensis CF311 ^{T}	76.1	69.5	25.2	0.94979
S. dokdonensis DSM 21029 ^{T}	72.9	63.3	22.3	0.94930
S. paucimobilis NBRC 13935 ^{T}	72.9	63.6	22.4	0.94423

ANIb — Average nucleotide identity, calculation based on BLAST+, http://jspecies.ribohost.com/jspeciesws/.
AAI — Amino acid identity, http://enve-omics.ce.gatech.edu/aai/.
Tetra z-score — statistical analysis of tetra-nucleotide usage patterns, http://jspecies.ribohost.com/jspeciesws/.
dDDH — Insilico DNA-DNA hybridisation score, http://ggdc.dsmz.de/ggdc.php.

Table 3
Whole cellular fatty acid composition of three novel *Sphingomonas* strains and related *Sphingomonas* species. Taxa; (1) L1A4; (2) 3CNBAF; (3) L3B27^T; (4) *Sphingomonas pituitosa* DSM 13101^T; (5) *S. azotifigens* DSM 18530^T; (6) *S. trueperi* DSM 7225^T; (7) *S. kyeonggiensis* JCM 18825^T; (8) *S. paucimobilis* KACC 10931^T. Only fatty acid percentages amounting 0.5% or higher are shown. t, (<0.5%); ND, not detected. All data were obtained in this study except *S. paucimobilis* KACC 10931^T data is taken from Kim et al. [24].

Fatty acids (%)	1	2	3	4	5	6	7	8
Saturated fatty acids								
$C_{14:0}$	0.5	0.6	0.6	0.8	1.7	0.4	0.6	t
$C_{16:0}$	27.9	29.3	28.7	16.4	29.4	16.1	19.5	14.5
$C_{18:0}$	1.4	1.5	1.7	1.8	1.7	3.0	1.0	t
Unsaturated fatty acids								
$C_{18:1}$ $\omega 5c$	0.7	0.7	0.7	1.0	0.6	1.2	0.5	2.2
$C_{18:1}$ $\omega 7c$ 11-methyl	7.4	7.4	5.1	0.9	6.4	ND	3.2	t
Hydroxy fatty acids								
$C_{14:0}$ 2-OH	6.5	5.5	5.9	10.0	7.7	7.2	7.1	5.9
$C_{16:0}$ 2-OH	0.6	0.5	0.8	t	0.5	t	0.6	t
Summed feature 3*	0.5	0.7	0.5	1.7	0.6	0.9	1.1	4.1
Summed Feature 8*	50.2	51.6	54.4	64.4	48.0	68.6	64.3	67.9

* Summed features represent groups of two fatty acids which could not be separated by GLC and the MIDI system. Summed feature 3 contained $C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 7c$, and feature 8 contained $C_{18:1}$ $\omega 6c$ and/or $C_{18:1}$ $\omega 7c$.

delineation [19,25], hence confirming the distinct taxonomic status of L3B27^T within the genus *Sphingomonas*.

To check for intraspecies diversity among the novel strains L3B27^T, 3CNBAF and L1A4, PCR based genomic DNA fingerprinting was performed using primer $(GTG)_5$. This result showed that strains L3B27^T, 3CNBAF and L1A4 had dissimilar genomic fingerprints among eachother indicating that they are not clones. Also, we could observe few bands commonly shared between the three novel strains which possibly demonstrate their species level relationships. In addition to this, the genomic fingerprinting profiles of the three novel strains are significantly different from their closest phylogenetic neighbours (Fig. 3).

The whole cell fatty acids of the strains L3B27^T, 3CNBF and L1A4 were dominated by summed feature 8 ($C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$) and $C_{16:0}$, a fatty acids profile similar to those reported for the genus *Sphingomonas* [7,12,43,56]. However, some qualitative and quantitative differences in fatty acid profiles which may help to differentiate the new taxon from its closest phylogenetic neighbours are highlighted in Table 3. The major polar lipid components of L3B27^T were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidyldimethylethanolamine (PDE) and a sphingoglycolipid (SGL). In addition, moderate to minor amounts of phosphatidyl choline (PC), phosphatidylmonomethylethanolamine (PME), diphosphatidylglycerol (DPG) and few unidentified lipids *i.e.* aminophospholipid (APL), aminophosphoglycolipid (APGL), aminolipid (AL), phospholipid (PL) and unknown lipid (UL) were also found (supplementary Fig. S1). Further, L3B27^T characterised by the presence of polyamines such as sym-homospermidine, spermidine and spermine as identified by their respective mass through LC–MS analysis (supplementary Fig. S2). Further, this analysis showed sym-homospermidine as the predominant polyamine in L3B27^T which was determined based on the comparative peak intensities of each individual polyamine [5,34]. L3B27^T contained Q10 as the major respiratory quinone. Thus, fatty acid analysis, polar lipid profile, quinone and polyamine pattern are well in line with the corresponding traits listed in the genus description of *Sphingomonas* [7,12,43,56].

The new taxon showed typical phenotypic characters which are shared by almost all species of the genus *Sphingomonas* such as Gram-strain-negative, obligate aerobe, non-spore forming, rod shaped cells and forms yellow pigmented colonies. The identified phenotypic characters which differentiates the new taxon from its nearest phylogenetic neighbours are shown in Table 4. Other char-

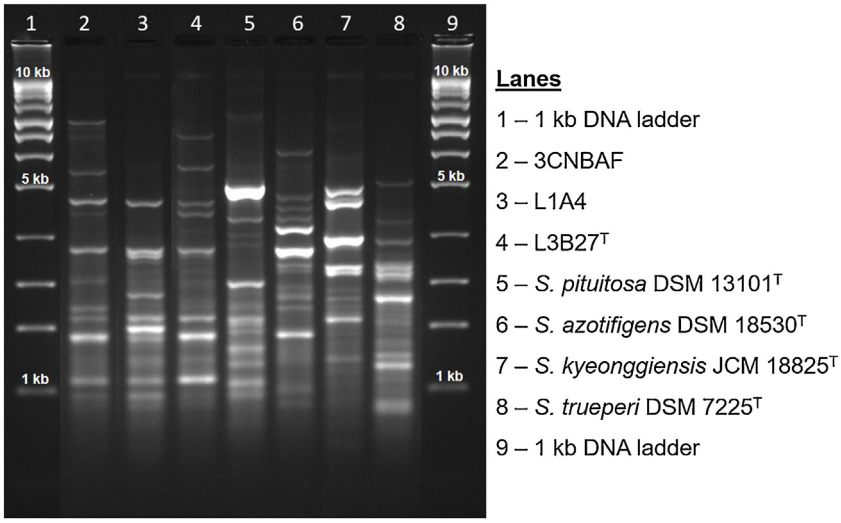

Fig. 3. Genomic fingerprint of the three novel *Sphingomonas* strains and its phylogenetically closest *Sphingomonas* type strains using $(GTG)_5$ primer.

Table 4
Differential phenotypic characteristics of three novel *Sphingomonas* strains and its phylogenetically closest type strains of the genus *Sphingomonas*. Taxa; (1) L1A4; (2) 3CNBAF; (3) L3B27^T; (4) *Sphingomonas pituitosa* DSM 13101^T; (5) *S. azotifigens* DSM 18530^T; (6) *S. trueperi* DSM 7225^T; (7) *S. kyeonggiensis* JCM 18825^T; (8) *S. paucimobilis* GIFU 2395^T (all data were taken from Yabuuchi et al. [52] except growth at different NaCl concentration is taken from Chen et al. [11] and antibiotic sensitivity is taken from Yabuuchi et al. [53]). Symbols; +, positive; –, negative; S, sensitive; R, resistant; NA, not available.

Phenotypic characters	1	2	3	4	5	6	7	8
Growth at 42 °C	+	+	+	–	–	–	–	NA
Growth at 2% NaCl	+	+	+	–	+	–	–	+
Growth at 3% NaCl	+	+	+	–	–	–	–	+
Hydrolysis of								
Starch	–	–	–	+	–	+	–	+
Urea	+	–	–	+	+	+	–	–
Utilisation as sole carbon source								
Trehalose	+	–	+	–	+	+	+	+
Raffinose	+	+	+	–	–	–	–	+
Utilisation as sole nitrogen source								
L-histidine	+	+	+	–	+	–	+	NA
L-leucine	+	+	+	–	+	+	+	NA
L-serine	+	+	+	–	+	+	+	NA
Acid Production (HiCarbohydrate Kit)								
D-arabinose	–	–	–	+	–	+	–	+
Mannose	–	–	–	+	+	+	–	+
Rhamnose	+	+	+	–	–	–	–	–
Antibiotic sensitivity tests								
Penicillin G (P) (10 units)	R	S	S	R	R	R	R	S
Cefazolin (Cz) (30 mcg)	R	R	S	R	R	R	R	R

acters including biochemical and physiological features of the new taxon are presented in detail in the species description section.

Genome insights for plant associated lifestyle

The final draft genome of L3B27^T has a single circular chromosome of 4,223,965 bp with G + C content of 66.18% (Fig. 4). The genome predicted to encode 3910 genes including 3860 protein coding genes and 50 RNA genes. The genome contains one copy of 16S rRNA, 23S rRNA and 5S rRNA genes, respectively. The 16S rRNA gene sequence of L3B27^T obtained from Sanger sequencing and draft genome shared 100% homology. A total of 2912 genes (74.47%) were predicted to have known functions while the rest were annotated as hypothetical or unknown proteins. The properties and the statistics of the L3B27^T genome are summarised in Table S2.

As shown in Table 5, the genome of L3B27^T contains extensive set of genes that probably relates its ability to associate with plant rhizosphere environments. For example, the annotated L3B27^T genome contains several genes responsible for flagellar motility and chemotaxis (Table 5). Notably, the genetic determinants of chemotaxis are located within the flagellar gene operon cluster as seen in other endophytic bacteria [47], suggesting that these gene features favourably help L3B27^T to get attracted and move towards plant exudates. To facilitate root attachment, the genome contained several genes coding for functions known for root adhesion; lectin type hemagglutinin [22], tad (tight adherence genes); *tadBC tadA cpaABCDE* [37] and T2SS-general secretion system; *gspDCI-HGLMEF gspJK gspE* [14]. Further, we could identify genes coding for polysaccharide biosynthesis (EPS), a major determinant for root attachment and biofilm formation [3]. In complement, we confirm the ability of L3B27^T to form biofilm through invitro microtiter plate assays Fig. S3a.

To cope with rhizosphere oxidative environments, L3B27^T is well equipped with genes to detoxify the reactive oxygen species generated while colonising the plant rhizosphere. This includes four superoxide dismutase (SOD); manganese SODs (2), Ferric SOD (1) and copper/zinc SOD (1) and three catalases.

Iron is an essential and limiting growth cofactor in the plant rhizosphere and L3B27^T has developed various mechanisms for efficient uptake of iron from the environment. It includes ferrichrome-iron receptors, iron uptake proteins and TonB dependent receptors. Notably, we could identify a marine type siderophore; vibrioferrin biosynthetic cluster (*pvsA pvsE pvsBC*) which is absent in the genomes of its closest type strains; *S. pituitosa* and *S. azotifigens*. The presence of a conjugative transfer protein TrbI and a mobile element protein in the downstream of the vibrioferrin biosynthetic cluster, suggests that this gene might have acquired through horizontal gene transfer [48]. Furthermore, several plant associated rhizobacteria uses siderophores as one of the ways to protect plants from various fungal pathogens. In this case, L3B27^T failed to inhibit tested plant fungal pathogens (data not shown) although it is positive for siderophore production (Fig. S3b), suggesting that L3B27^T probably use siderophores to scavenge iron.

To successfully establish in the plant rhizosphere environment, the L3B27^T has to utilise various plant derived compounds as nutrients. In this regard, the genome encodes complete set of genes involved in central carbohydrate metabolism suggesting that L3B27^T can utilise carbohydrates as nutrients. Further, the genome annotated to contain several genes involved in either transport or

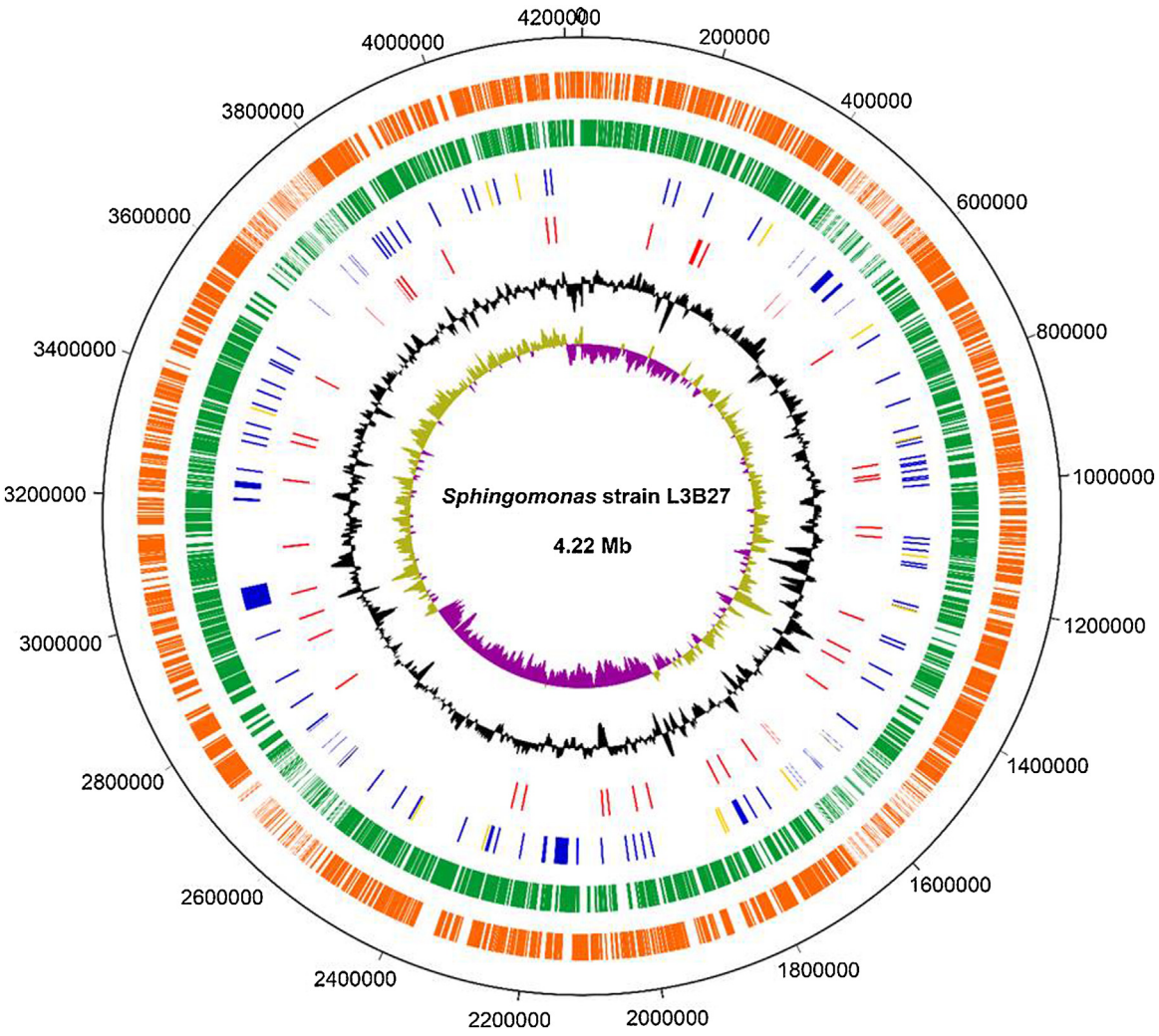

Fig. 4. Genomic representation of the novel *Sphingomonas* strain L3B27^T. Rings from inside; Ring 1-GC content, Ring 2- C skew, Ring 3-tRNA genes, Ring 4-Plant associated genes (Blue) and brackish adaptation genes (yellow), Ring 5-Predicted CDS in the counter-clockwise and Ring 6-predicted CDS in clockwise direction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

utilisation of various plant derived sugars, amino acids and organic acids which were later confirmed phenotypically (Table S3, S4). Besides this information, the L3B27^T genome also harbours several genes that are likely involved in the degradation of plant derived aromatic compounds (Table S5). Also, the genome contains higher percentages of carbohydrate active enzymes (~40%) which were distributed among several families of glycosyl hydrolases (GHs) including GHs specific for plant polymer degradation. For example, the genome codes for enzymes; β-xylosidase (GH39 and GH43; EC 3.2.1.37) and endo-1,4-β-xylanase (GH10; EC 3.2.1.8) which are likely involved in the xylan degradation, an abundant plant polysaccharide [16,36,38]. Also, the genome contain genes coding for D-xylose monosaccharides degradation; D-xylose 1-dehydrogenase (EC 1.1.1.175) and xylonolactonase (EC 3.1.1.68). Altogether, these genome results certainly suggest that L3B27^T is well adapted to sustain in the plant rhizosphere.

Protein secretion systems play a central role in the interaction of bacteria with their surrounding environments, in particular when they (symbiotic or pathogenic) interact with their eukaryotic hosts [21,50]. The L3B27^T genome was identified to contain complete set of genes coding for general secretion pathway (gsp); *gspDCIHGLMEF gspJK gspE, sec* elements *secY secE secA secG secFD* and twin-arginine translocation components *tatABC*, where these systems are majorly involved in the transport of proteins across the cytoplasmic membrane. The genome also contains certain components of the type III and IV secretion systems, however genes related to the needle formation, translocation components and the effector proteins were absent, indicating that type III and IV secretion systems are incomplete, therefore may not fully functional. In addition to this, we could identify three LuxR solos, a quorum sensing regulator in the genome, but no genes encoded for acyl homoserine lactone (AHL) were found. The presence of LuxR solos indicates that L3B27^T can respond to exogenous or endogenous AHLs produced by the neighbouring cells or non-AHL signal like molecules produced by the plants [46].

The genome does not encode for genes responsible for nitrogen fixation, 1-aminocyclopropane carboxylate (ACC) deaminase production, phosphate solubilisation and production of secondary metabolites known to kill bacterial and fungal pathogens. This was further confirmed by invitro plate assays where the results were negative for the above plant growth promoting properties. However, the presence of a tryptophan independent indole acetic acid (IAA) production pathway indicates that L3B27^T might use this pathway to produce the phytohormone. In contrast, we found that strain L3B27^T able to produce 45.75 ± 2.31 μg/ml of IAA in presence of tryptophan.

Next, we searched for gene features that could enable L3B27^T to survive in brackish environments. The genome contained nine

Table 5
Putative gene features identified in L3B27^T genome related to plant associated life style and brackish water adaptation.

Functions	Components	Associated gene no.	Function in strain L3B27^T
Tricarboxylic acid cycle	Catabolic genes	14	Carbon metabolism from plant exudates
Entner-Doudoroff pathway	Catabolic genes	12	
Pentose cycle	Catabolic genes	7	
Rhizosphere nutrient uptake	Rhizosphere transporters regulated by PTS and GPH	14	
Chemotaxis	*cheDYBRWA* and *cheV* and other chemotaxis related genes	32	Sensing chemical stimulus and direct motility
Motility	Flagellar structural and regulatory genes	40	Motility
Root colonisation	Hemagglutinin (Lectin)	1	Adhesion to the plant root
	EPS	18	
	Tad adherence	9	
Transporters	MFS genes	38	Transporters involved in bacterium rhizosphere interaction
	RND genes	19	
	ABC genes	59	
Acetoin and 2,3-butanediol synthesis	acetolactate synthase and acetoin dehydrogenasem	9	Plant growth regulators synthesis and catabolism
IAA	Tryptophan independent pathway	14	
Secretion systems	Type I, Type II	28	Transport of biocontrol molecules
Iron uptake	Vibrioferrin siderophore biosynthesis, TonB dependent receptors	69	Ion uptake to compete with pathogens
Detoxification and ionic balancing	Glutathione S-transferase, peroxiredoxin, glutaredoxin, SOD, peroxidase, catalase	40	Detoxification of ROS response by the host plant
Brackish adaptation	Ion channels, BetT, BetA and Aquporin Z	15	Involved in osmoregulation

PTS, Phosphotransferase System; MFS, The major facilitator superfamily; RND, Resistance Nodulation-Division; IAA, indole acetic acid; SOD, superoxide dismutase; ROS, Reactive oxygen species; ABC transporter, ATP binding cassette transporter; che, Chemotaxis.

genes coding for Na+ based ion channels which includes three Na+/H+ antiporter genes of which one is NhaA type Na+ and H+ antiporter and one Na+ dependent nucleoside transporter NupC. Further, the L3B27^T genome contains genes coding for glutathione production, choline dehydrogenase (BetA), a high affinity choline uptake protein (BetT) and aquaporin Z which are known previously to play an important role in osmotic stress responses [8,51].

In summary, the results obtained from genotypic including genome, phenotypic and chemotaxonomic analysis clearly differentiated the novel strains from its closest type strains *S. pituitosa* DSM 13101^T, *S. azotifigens* DSM 18530^T and *S. trueperi* DSM 7225^T. Thus, based on the polyphasic approach, we describe a novel *Sphingomonas* species for which the name *S. pokkalii* sp. nov (type strain L3B27^T = KCTC 42098^T = MCC 3001^T) is proposed. Also, the novel taxon possesses several phenotypic traits that include utilisation of various plant derived compounds, biofilm formation and siderophore production, making it an efficient plant associated beneficial rhizobacteria. Further, the draft genome analysis provides insights into the genetic features that supports its plant associated lifestyle and brackish adaptation.

Species description of Sphingomonas pokkalii sp. *nov*

Sphingomonas pokkalii (pok.ka'li.i. N.L. gen. n. *pokkalii* of pokkali (a variety of rice).

Cells are gram negative, rod-shaped and motile with strict aerobic lifestyle. Colonies are yellow pigmented, circular, entire, convex and smooth with 0.5–0.8 mm in diameter after 4 days at 30 °C in R2A agar medium. Growth occurs in Luria Bertani agar, nutrient agar, trypticase soya agar, but not in ZoBell marine agar. Grows at temperature ranging from 18 to 42 °C, but not at 4 °C and 45 °C (optimal growth occurs at 28–30 °C). Tolerates up to 3% NaCl (w/v) (optimal at 0–0.5%). Able to grow in different seawater concentrations (50 and 100%) amended in R2A broth medium. Cells form long filaments under higher NaCl and seawater concentrations. Positive for catalase but negative for oxidase, anaerobic growth, indole production, arginine dihydrolase, lysine decarboxylase, nitrate reduction, methyl red and Voges Proskauer (MR-VP) tests. Positive hydrolysis for esculin, tributyrin and Tween 80 whereas negative hydrolysis for casein, DNA, starch, pectin, xylan, chitin, carboxymethyl cellulose, and gelatin. Urease production showed variable reaction. In HiCarbohydrate kit, galactose, rhamnose and xylose showed positive acid production whereas adonitol, L-arabinose, D-arabinose, arabitol, cellobiose, dextrose, dulcitol, erythritol, fructose, glycerol, inositol, inulin, lactose, maltose, mannitol, mannose, melibiose, α methyl D-glucoside, α methyl D-mannoside, melezitose, raffinose, salicin, sodium gluconate, sorbitol, sorbose, sucrose, trehalose and xylitol showed negative acid production. ONPG test is positive indicating the presence of β-galactosidase, but citrate and malonate is not utilised. L-arabinose, D-cellobiose, D-dextrose, D-fructose, lactose, D-maltose, raffinose, succinic acid, xylose are utilised as sole carbon sources whereas citric acid, malic acid, D-mannitol and rhamnose are not utilised. Trehalose utilisation showed variable reaction. L-alanine, L-histidine, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan and L-valine are utilised as sole nitrogen sources. Susceptible to the following antibiotics (μg/disc): amoxyclav (30), ampicillin (10), carbenicillin (100), cefazolin (30) (strains L1A4 and 3CNBAF was resistant), chloramphenicol (30), ciprofloxacin(5), co-trimaxazole (25), doxycycline hydrochloride (30), erythromycin (15), gentamicin(10), kanamycin (30), linezolid (30), nalidixic acid (30), nettilin (30), ofloxacin (5), penicillinG (10 units) (strain L1A4 was resistant), rifampicin (5), tetracycline (30), vancomycin (30), polymixin B (300 units) and resistant to cinoxacin (100) (strain 3CNBAF was sensitive), clindamycin (2), oxacillin (1), methicillin (5) and streptomycin (10). The major fatty acids were summed feature 8 ($C_{18:1}\omega7c/C_{18:1}\omega6c$) and $C_{16:0}$ and the complete cellular fatty acid profile is given in Table 3. Other chemotaxonomy properties such as polar lipid profile, respiratory quinone and polyamines are given in the main text. The strain possessed plant growth promoting properties such as production of Indole acetic acid, siderophore production, biofilm formation and utilisation of various sugars, amino acids and organic acids.

The type strain L3B27^T (=KCTC 42098^T = MCC 3001^T) was isolated from the rhizosphere of a saline tolerant pokkali rice variety (chettivirippu) grown in Chellanam, Kerala, India. The DNA G + C content of the type strain L3B27^T is 66.18 mol%.

Digital Protologue Taxonumber is TA00635

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.syapm.2019.02.003.

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