Average nucleotide identity of genome sequences supports the description of *Rhizobium lentis* sp. nov., *Rhizobium bangladeshense* sp. nov. and *Rhizobium binae* sp. nov. from lentil (*Lens culinaris*) nodules

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Rhizobial strains isolated from effective root nodules of field-grown lentil (*Lens culinaris*) from different parts of Bangladesh were previously analysed using sequences of the 16S rRNA gene, three housekeeping genes (*recA*, *atpD* and *glnII*) and three nodulation genes (*nodA*, *nodC* and *nodD*), DNA fingerprinting and phenotypic characterization. Analysis of housekeeping gene sequences and DNA fingerprints indicated that the strains belonged to three novel clades in the genus *Rhizobium*. In present study, a representative strain from each clade was further characterized by determination of cellular fatty acid compositions, carbon substrate utilization patterns and DNA–DNA hybridization and average nucleotide identity (ANI) analyses from whole-genome sequences. DNA–DNA hybridization showed 50–62 % relatedness to their closest relatives (the type strains of *Rhizobium etli* and *Rhizobium phaseoli*) and 50–60 % relatedness to each other. These results were further supported by ANI values, based on genome sequencing, which were 87–92 % with their close relatives and 88–89 % with each other. On the basis of these results, three novel species, *Rhizobium lentis* sp. nov. (type strain BLR27T =LMG 28441T =DSM 29286T), *Rhizobium bangladeshense* sp. nov. (type strain BLR175T =LMG 28442T =DSM 29287T) and *Rhizobium binae* sp. nov. (type strain BLR195T =LMG 28443T =DSM 29288T), are proposed. These species share common nodulation genes (*nodA*, *nodC* and *nodD*) that are similar to those of the symbiovar *viciae*.

Rhizobia are nodule-forming, nitrogen-fixing bacteria that belong to the bacterial phylum *Proteobacteria*. Rhizobia can satisfy the nitrogen requirement of legumes by effective symbiosis with these plants and are therefore important bacteria for supporting plant growth and for environmental protection. About 180 species of nodule-forming bacteria in 12 different genera have been described. *Rhizobium* is an important genus of rhizobia, and at least 76 species are currently included in this genus (http://www.bacterio.net/rhizobium.html). Among agricultural legume crops, lentil (*Lens culinaris*) is one of the oldest and remains very popular all over the world; it forms an effective symbiosis with rhizobia. *Rhizobium leguminosarum* symbiovar *viciae* (RlV) is the main symbiont of the legume tribe Vicieae (Hou et al., 2009; Laguerre et al., 2003; Mutch & Young, 2004; Santillana et al., 2008; Tian et al., 2010; and many others), to which lentils belong, although other species, including *Rhizobium pisi*, *R. fabae*

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; ML, maximum-likelihood.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *atpD*, *recA* and *glnII* gene sequences of strains BLR27T, BLR175T and BLR195T are JN648905, JN648931 and JN648932 (16S rRNA gene), JN648941, JN648967 and JN648968 (*atpD*), JN649031, JN649057 and JN649058 (*recA*) and JN648976, JN648979 and JN648980 (*glnII*), respectively. The whole-genome sequences of the three strains are available at http://www.ebi.ac.uk/ena/data/view/PRJEB7125.

Five supplementary tables are available with the online Supplementary Material.
and R. laguerreae (Ramirez-Bahena et al., 2008; Saïdi et al., 2014; Tian et al., 2008), have also been described. Previous studies on rhizobia from lentil root nodules from different geographical locations have revealed that Rhizobium is the major symbiont of lentil (Geniaux & Amarger, 1993; Hynes & O’Connell, 1990; Laguerre et al., 1993; Materon et al., 1995; Moawad & Beck, 1991). However, in our previous studies (Rashid et al., 2012, 2014), we identified three novel rhizobial clades or lineages in Bangladesh by multilocus sequence analysis and phenotypic and DNA fingerprint analyses. DNA–DNA hybridization (DDH) is an important experimental method to detect relatedness between two genome sequences, and a DDH value of 70% is regarded as the threshold value for bacterial species demarcation (McCarthy & Bolton, 1963). Wayne et al. (1987) agreed that ‘the complete deoxyribonucleic acid (DNA) sequence would be the reference standard to determine phylogeny and that phylogeny should determine taxonomy’, which was an impracticable goal at the time, but is increasingly feasible (Chan et al., 2012). The approach known as average nucleotide identity (ANI) (Konstantinidis & Tiedje, 2005) provides relatedness information for prokaryotes at the whole-genome level. ANI values equal to 95–96%, calculated from pairwise comparisons of shared sequences between two genomes, provide a value equivalent to DDH values of 70% (Chan et al., 2012; Goris et al., 2007; Konstantinidis & Tiedje, 2005; Wayne et al., 1987).

Rhizobial strains were isolated from field-grown lentil root nodules from different parts of Bangladesh in 2009. Detailed descriptions of the strains (isolation localities, isolation procedure; identities; phylogenetic and population analysis; DNA fingerprint analysis and phenotypic characterization) were provided by Rashid et al. (2012, 2014). DNA fingerprint analysis using ERIC-PCR, phylogeny and population analysis of recA, atpD and glnII genes strongly suggested that the three clades formed by the strains were clearly distinct from all described species of the genus Rhizobium and represent three novel species. In present study, we extended the phylogeny of the previously described 15 strains by including recently described rhizobial species. Three strains from these three lineages (BLR27T, BLR175T and BLR195T from lineages I, II and III, respectively) were further characterized in this study by cellular fatty acid composition analysis, DDH and ANI to determine whether the proposal of novel species was justified.

We previously described three novel lineages in the genus Rhizobium from effective root nodules of lentils from Bangladesh (Rashid et al., 2012). Since then, several additional species have been described in this genus. Therefore, in present study, the 16S rRNA, recA and atpD gene sequences from the 15 strains from our previous study were compared with those of members of recently described species to assess their taxonomic status. The sequences were obtained from the NCBI and aligned with CLUSTAL_X (Thompson et al., 1997) in BioEdit (Hall, 1999). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (ML) (Rogers & Swofford, 1998) methods in MEGA version 6 (Tamura et al., 2013). For sequence evolution, the general time reversible (GTR) model with gamma distribution was used in ML analysis. Bootstrap support for each node was evaluated with 1000 replicates. Trees were rooted using the genus Mesorhizobium as an outgroup. The 16S rRNA gene sequences from strains of the proposed novel species were very similar and clustered with those of Rhizobium etli CFN 42T, R. phaseoli ATCC 14482T, R. fabae CCBAU 33202T, R. pisi DSM 30132T, R. leguminosarum USDA 2370T and R. laguerreae FB 206T (Fig. 1). The 16S rRNA gene did not provide a clear taxonomic identification, since the sequences were >99% identical among the novel strains and representatives of more than one closely related species. Analysis of recA, atpD and glnII genes and their concatenated sequences from our previous study (Rashid et al., 2012) and the concatenated sequences of the recA and atpD genes (Fig. 2) revealed that the proposed species formed three separate clades/lineages and that the closest relatives were R. etli and R. phaseoli. Moreover, Rhizobium sp. strain ESC 1110, isolated from Phaseolus vulgaris from Hispaniola (Díaz-Alcántara et al., 2014), was closely related to strain BLR27T (Fig. 2).

The phylogeny of the housekeeping genes recA and atpD was first used by Gaunt et al. (2001) and the glnII gene by Turner & Young (2000), Stepkowski et al. (2005) and Vinuesa et al. (2005) to delineate the phylogeny of rhizobia and related bacteria with more confidence than is possible with the 16S rRNA gene alone. These markers have been used widely and successfully in many studies of rhizobial diversity since then, although additional housekeeping genes may improve the reliability of this approach further (Martens et al., 2007; Vinuesa, 2010). Based on a phylogenetic analysis of these three housekeeping protein-coding genes, the lentil isolates fell into three clades that were distinct enough from each other and from known species to suggest that they might represent three novel species. The phylogeny of housekeeping genes is particularly useful for placing multiple novel isolates in relation to those described previously, as in this instance. It should be noted that, although the housekeeping gene phylogenies indicate that the lentil rhizobia from Bangladesh form three distinct lineages, their nodulation gene sequences do not reflect these lineages. Instead, the majority of strains, regardless of clade, share identical sequences for three genes (nodA, nodC and nodD) involved in nodulation of the host plant (Rashid et al., 2012). These, and the variants found in the remaining strains, fall within the range of variation characteristic of symbiovar viciae. The symbiovar is a key attribute for the description of rhizobia: strains belonging to a symbiovar have similar nodulation genes and nodulate a similar range of hosts (Rogel et al., 2011). To observe the host range of proposed species for nodule formation, cross-inoculation tests were performed with pea (Pisum sativum) and lathyrus (Lathyrus sativa). Randomly selected strains from the three proposed novel species were able to form nodules with both pea and lathyrus, suggesting that the proposed novel species are typical members of symbiovar
Fig. 1. ML tree reconstructed from partial 16S rRNA gene sequences. Bootstrap values ≥ 70% are indicated for each node (1000 replicates). Bar, 0.01 substitutions per nucleotide position.
Fig. 2. ML tree reconstructed from concatenated sequences of the **atpD** and **recA** genes. Bootstrap values ≥ 70% are indicated for each node (1000 replicates). Bar, 0.05 substitutions per nucleotide position.
viciae (Rashid et al., 2012). Importantly, strains that share the same symbiovar need not belong to the same species, because the nodulation genes are part of the accessory genome (Young et al., 2006) and have frequently been subject to horizontal gene transfer within and between species (Young & Wexler, 1988). In this instance, all the lentil symbionts, regardless of species, belong to symbiovar viciae (Rashid et al., 2012).

Genetic diversity of the strains within each of the three clusters was assessed by high-resolution ERIC-PCR, showing that the strains that belong to the same cluster were not clonal (Rashid et al., 2012). For describing novel rhizobial species, uncorrected genetic distances (similarity levels) are an important parameter. Sequences of the recA and atpD genes of the three proposed species differed by 3.8–11.4 % from those of the type strains of all other species in this clade of Rhizobium (Table S1, available in the online Supplementary Material). On the other hand, differences among strains within each species never exceeded 1.0 % (Table S2), even though ERIC-PCR demonstrated that all the strains were genetically distinct (Rashid et al., 2012; Tables S1 and S2).

High-quality DNA was prepared using the method of Wilson (1987) with minor modifications (Cleenwerck et al., 2002). DDH was performed using a microplate method at 47.8 °C with photobiotin-labelled probes as described previously (Goris et al., 2007) using an HTS7000 Bio Assay Reader (PE Applied Biosystems) for fluorescence measurements. The DNA G+C content was determined by HPLC as described previously (Mesbah et al., 1989). DDH experiments were conducted with the type strains of Rhizobium etli and R. phaseoli, since these two species were very close to the novel strains in phylogenetic analyses. The results of the DDH experiments are shown in Table 1. Strain BLR27T (the proposed type strain of the species that makes up clade I, Rhizobium lentis sp. nov.) showed 50 and 56 % DNA relatedness to the type strains of Rhizobium etli and R. phaseoli, respectively. It showed 60 % relatedness to strain BLR175T (the proposed type strain of the proposed species that makes up clade II, Rhizobium bangladeshense sp. nov.) and 50 % DNA relatedness to strain BLR195T (the proposed type strain of the species that makes up clade III, Rhizobium biniae sp. nov.). Strain BLR175T showed 53 % relatedness to BLR195T. Overall, the proposed type strains (BLR27T, BLR175T and BLR195T) showed 50–62 % relatedness to the type strains of the most closely related species and 50–60 % relatedness to each other. The DNA G+C contents of the novel strains were 61.1, 60.9 and 61.4 mol% for strains BLR27T, BLR175T and BLR195T, respectively. These values are within the range reported for the genus Rhizobium (Jordan, 1984).

ANI is the best approach to determine genetic relatedness between two genomes, because this method evaluates a large number of genes in its calculation, including slowly and quickly evolving genes, and thus minimizes the effect of variable evolutionary rates or horizontal gene transfer events (Konstantinidis & Tiedje, 2005). Genomic DNA was extracted from strains grown in TY medium (Beringer, 1974) using PowerSoil DNA isolation kits (MoBio), and then fragmented, barcoded, quantified and run as part of a batch of eight genomes on a 318 chip on an Ion Torrent PGM using the manufacturer’s recommended protocols (Thermo Fisher). Each genome was assembled using the Newbler GS De novo assembler version 2.8 (Roche Diagnostics) with default parameter values. ANI was calculated within the JSpecies software (Richter & Rosselló-Móra, 2009). The Nucleotide MUMmer algorithm (NUCmer) was used, with default parameter settings, to calculate the ANI by subtracting the similarity errors from the alignment length (Kurtz et al., 2004; Richter & Rosselló-Móra, 2009). Genomes were compared with each other, with genome assemblies obtained using the same methods for R. pisi DSM 30132T and R. fabae CCBAU 33202T (unpublished data), and with complete genome assemblies downloaded from NCBI for the following strains: R. etli CFN 42T (accession no. GCA_000029045), R. phaseoli CIAT 652 (GCA_000020265), R. leguminosarum 3841 (GCA_000009265), R. leguminosarum WSM 1325 (GCA_000023185) and Rhizobium sp. WSM 2304 (GCA_000021345). Ion Torrent sequencing yielded sequence of 155, 264 and 237 Mbp from strains BLR27T, BLR175T and BLR195T, respectively, corresponding to 27- to 49-fold coverage, so it can be expected that virtually all the genomic sequence is included. Assembly resulted in 140, 89 and 187 contigs (>100 bp) with N50 sizes of 229, 286 and 173 kb, respectively. Pairwise ANI was calculated between these genomic sequences, and with other strains in the R. leguminosarum species complex for which genome data were available.

### Table 1. Genetic relatedness measured by DDH between strains representing the novel clades and type strains of the most closely related species

<table>
<thead>
<tr>
<th>Strain</th>
<th>DNA G+C content (mol%)</th>
<th>DNA–DNA relatedness (%) with:</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. R. lentis sp. nov. BLR27T</td>
<td>61.1</td>
<td>(100)</td>
</tr>
<tr>
<td>2. R. bangladeshense sp. nov. BLR175T</td>
<td>61.0</td>
<td>60.9</td>
</tr>
<tr>
<td>3. R. biniae sp. nov. BLR195T</td>
<td>61.5</td>
<td>50.4</td>
</tr>
</tbody>
</table>
(Table 2). Each ANI was calculated in both directions, but the results never differed by more than 0.03 %. ANI values ranged from 87.27 to 92.39 %, with this highest value being between strain BLR195T and R. phaseoli CIAT 652. All these values are well below 96 %, which is an accepted value as the species boundary, equivalent to a DDH of 70 % (Richter & Rosselló-Móra, 2009). Hence, each of the three novel strains represents a distinct species, and they are different from all closely related species described previously.

Different phenotypic characteristics, i.e. colony size, growth on LB medium, acid/alkali production, tolerance of NaCl, temperature and pH and antibiotic sensitivity, were determined following the protocols described by Rashid et al. (2012). Utilization of different carbon substrates by the novel strains and close relatives was determined using Biolog GENIII following the manufacturer’s instructions and the results are given in Table S3. Carbon and nitrogen substrate utilization patterns of the novel strains differed from those of their close relatives and among themselves. The cellular fatty acid compositions of the type strains of the proposed novel species were analysed after growth on YEMA plates at 28 °C for 3 days. Cells were saponified and transmethylated as described by Kuykendall et al. (1988) and fatty acid methyl esters were separated by using the Sherlock microbial identification system (RTSBA6; MIDI) and an Agilent (model 680N) gas chromatograph; profiles were determined at the DSMZ, Braunschweig, Germany. The results of fatty acid analysis are given in Table S4. The type strains of the proposed species and their close relatives shared some common fatty acids, 16 : 0, 18 : 0, 16 : 0 3-OH, 19 : 0 cyclo ω8c, summed feature 2 (one or more of 12 : 0 aldehyde, unknown ECL 15.489, 14 : 0 3-OH and iso-16 : 1 I) and summed feature 8 (18 : 1ω7c and/or 18 : 1ω6c), but the amounts were different between the strains. Moreover, iso-15 : 0 2-OH and summed feature 3 (16 : 1ω7c and/or 16 : 1ω6c) were found in two of the proposed novel species. Phenotypic characteristics of strains belonging to the three proposed species and their close relatives are given in Table S5. Strains of R. binae sp. nov. and most strains of R. bangladeshense sp. nov. were able to grow at pH 10, and also in 0.5 % NaCl, unlike most strains of R. lentis sp. nov. Furthermore, all strains of R. lentis sp. nov. and some strains of R. bangladeshense sp. nov. showed resistance to ampicillin, in contrast to their close relative (R. etli) with (R. etli; Segovia et al., 1993).

We have selected one representative strain from each clade that was identified in the housekeeping gene phylogeny, and demonstrate through the use of DDH and ANI analysis that they do in fact meet the standard criteria for distinct species, since all DDH values were below 70 % and ANI values were below 96 %. DDH has been the standard method for bacterial species demarcation for the last 50 years (McCarthy & Bolton, 1963; Tindall et al., 2010; Wayne et al., 1987), but it has major limitations. It is time-consuming, laborious and hard to standardize between laboratories. An increasingly significant limitation, as more species are described, is that DDH requires a laboratory comparison with all possible close relatives. DDH was developed before genome sequencing became feasible, but sequence-based methods have the potential to provide more reliable information more easily. The calculation of ANI from genome sequence data has been shown to give comparable results to DDH, with a species boundary at around 96 % (Goris et al., 2007; Konstantinidis & Tiedje, 2005). Our study does not test the accuracy of this boundary, since all ANI values were much lower than this, but it does provide further evidence that low ANI values can be used as an effective substitute for DDH when establishing that strains do not belong to the same species. In this study, the two methods provide consistent evidence that three novel species are involved in lentil nodulation in Bangladesh, for which we propose the names Rhizobium lentis sp. nov., Rhizobium bangladeshense sp. nov. and Rhizobium binae sp. nov.

**Table 2.** ANI (%) between strains representing the novel clades and the most closely related sequenced members of the R. leguminosarum complex

<table>
<thead>
<tr>
<th>Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>R. lentis sp. nov. BLR27T</td>
<td>100</td>
<td>89.70</td>
<td>88.64</td>
</tr>
<tr>
<td>R. bangladeshense sp. nov. BLR175T</td>
<td>89.26</td>
<td>100</td>
<td>88.51</td>
</tr>
<tr>
<td>R. binae sp. nov. BLR195T</td>
<td>88.62</td>
<td>88.51</td>
<td>100</td>
</tr>
<tr>
<td>R. etli CFN 42T</td>
<td>89.04</td>
<td>88.64</td>
<td>89.28</td>
</tr>
<tr>
<td>R. phaseoli CIAT 652</td>
<td>88.56</td>
<td>88.25</td>
<td>92.39</td>
</tr>
<tr>
<td>R. fabae CCBAU 33202T</td>
<td>89.90</td>
<td>88.64</td>
<td>88.81</td>
</tr>
<tr>
<td>R. pisi DSM 30132T</td>
<td>88.82</td>
<td>88.18</td>
<td>88.46</td>
</tr>
<tr>
<td>R. leguminosarum 3841</td>
<td>88.33</td>
<td>87.27</td>
<td>88.28</td>
</tr>
<tr>
<td>R. leguminosarum WSM 1325</td>
<td>88.01</td>
<td>87.39</td>
<td>88.06</td>
</tr>
<tr>
<td>Rhizobium sp. WSM 2304</td>
<td>88.09</td>
<td>87.86</td>
<td>88.55</td>
</tr>
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</table>

**Description of Rhizobium lentis sp. nov.**

*Rhizobium lentis* (len’tis. L. gen. n. lentis referring to the lentil and, in botany, to *Lens*, the plant genus from which the first strains were isolated).

Cells are Gram-negative-staining, aerobic, non-sporulating and rod-shaped. The optimum growth temperature is 28 °C at pH 7. Colonies are circular, convex and creamy white on YEMA medium. Strains grow at 12–37 °C but can survive at 4 °C. Strains grow well at pH 5.5–8.2 and are sensitive to 0.5 % NaCl in YEMA medium. Most known strains are resistant to ampicillin, kanamycin and nalidixic acid. Strains do not tolerate tetracycline and do not show any growth on LB medium. The fatty acid composition of the type strain is iso-15 : 0 2-OH, 16 : 0, 16 : 0 3-OH, 18 : 0, 19 : 0 cyclo ω8c and summed features 2 (one or more of 12 : 0 aldehyde, unknown ECL 15.489, 14 : 0 3-OH and iso-16 : 1 I), 3 (16 : 1ω7c and/or 16 : 1ω6c) and 8 (18 : 1ω7c and/or 18 : 1ω6c). In the Biolog III system, the type strain can utilize lactose, methyl β-D-glucoside, D-sorbitol, D-mannitol,
D-arabitol, glycerol, D-fructose 6-phosphate, L-aspartic acid, D-glucuronic acid, mucic acid, D-lactic acid methyl ester, L-lactic acid, 1-histidine, β-hydroxy-DL-butyric acid, D- and L-malic acid, acetic acid and formic acid. The type strain is unable to utilize maltoose, trehalose, cellobiose, gentiobiose, sucrose, raffinose, α-D-glucose, turanose, melibiose, mannose, galactose, 3-methyl glucose, inosine, D-aspartic acid, glycyrl-L-proline, L-alanine, L-arginine, L-serine, pectin, D-saccharic acid, D-aspartic acid, glycyl-L-proline, L-alanine, L-arginine, D- and L-malic acid, acetic acid and propionic acid. The type strain can grow in the presence of lincomycin, tetrazolium violet, tetrazolium blue, nalidixic acid, lithium chloride or sodium butyrate.

The type strain is BLR27T (=LMG 28441T = DSM 29286T). The DNA G+C content of the type strain is 61.1 mol%. The type strain was isolated from effective nodules of *Lens culinaris* from Mohammepur, Khula district, Bangladesh. Other strains (BLR62, BLR99, BLR129, BLR153 and BLR154) were isolated from different parts of Bangladesh.

**Description of Rhizobium binae sp. nov.**

*Rhizobium binae* (bi’nae. N.L. gen. fem. n. *binae* arbitrary name formed from the abbreviation for the Bangladesh Institute of Nuclear Agriculture, the research institute where the first steps to isolate the species were taken).

Cells are Gram-negative-staining and rod-shaped. Colonies are circular, convex and creamy white on YEMA medium. The optimum temperature for growth is 28 °C at pH 7, but strains grow well at 37 °C. Strains survive at pH 5.5–10 and tolerate 1 % NaCl in YEMA. They are very sensitive to ampicillin and resistant to kanamycin and nalidixic acid. Strains do not tolerate tetracycline and do not show any growth on LB medium. The fatty acid composition of type strain is iso-15:0 2-OH, 16:0, 16:0 3-OH, 18:0, 18:1o9c, 18:0 3-OH, 11-methyl 18:1o7c, 19:0 cyclo 11con and summed features 2 (one or more of 12:0 aldehyde, unknown ECL 15.489, 14:0 3-OH and iso-16:1 I) and 8 (18:1o7c and/or 18:1o6c). The type strain can utilize dextrin, maltose, trehalose, cellobiose, gentiobiose, sucrose, raffinose, α-D-glucose, turanose, lactose, D-fructose, melibiose, methyl β-D-glucoside, salicin, N-acetyl-D-galactosamine, D-mannose, D-galactose, D-mannitol, D-sorbitol, D-arabitol, glycerol, D-glucose 6-phosphate, D-fructose 6-phosphate, D-alanine, L-aspartic acid, L-histidine, L-pyroglutamic acid, quinic acid, D-saccharic acid, methyl pyruvate, L-lactic acid, D- and L-malic acid, bromosuccinic acid, β-hydroxy-DL-butyric acid and acetic acid. The type strain fails to utilize N-acetyl-D-mannosamine, 3-methyl glucose, inosine, glycyrl-L-proline, L-arginine, D-galacturonic acid, D-glucuronic acid, glucuronamide, p-hydroxyphenylacetic acid, D-lactic acid methyl ester, α-ketoglutaric acid, Tween 40, propionic acid and formic acid. The type strain can grow in the presence of lincomycin and potassium tellurite but not with 1 % sodium lactate, troleandomycin, lithium chloride or sodium butyrate.

The type strain is BLR195T (=LMG 28443T = DSM 29288T). The DNA G+C content of the type strain is 61.5 mol%. The type strain was isolated from effective nodules of *Lens culinaris* from Sarishadi, Feni district, Bangladesh. The type strain and strains BLR228 and BLR235 were isolated from south-east Bangladesh.

Three novel species of *Rhizobium* that nodulate lentil

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