Genome sequence of “Leucobacter massiliensis” sp. nov. isolated from human pharynx after travel to the 2014 Hajj

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Abstract

“Leucobacter massiliensis” strain 122RC15T sp. nov. is a new species within the genus Leucobacter. The genome of this strain is described here. It was isolated from the pharynx of a 76-year-old Algerian female after travelling from the 2014 Hajj. “Leucobacter massiliensis” is a Gram-positive, aerobic bacillus. Here we describe the features including complete genome and annotation of this strain. The 3,136,406-bp long genome contains 2,797 protein-coding genes and 49 RNA genes.

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Materials and methods

Strain identification
A pharyngeal sample was obtained from a healthy pilgrim after travelling to the 2014 Hajj. The pharyngeal sample was processed as previously described [1]. This strain was isolated by cultivation on Chocolate agar PolyViteX (bioMérieux, Marcy l’Étoile, France) under a 5% CO2 atmosphere at 37°C after 1 day of incubation. For bacterial identification, matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF-MS) protein analysis was carried out as previously described [13]. The 122RC15 spectra were imported into MALDI Biotyper 3.0 software (Bruker Daltonics, Leipzig, Germany) and analysed by standard parameter matching (with default parameter settings) against 7765 spectra of bacteria. From the resulting scores, the tested strain may or may not be identified compared with the instrument’s database; a score of ≥2 with a validly published species enabled identification at the species; a score ≥1.7 and <2 allows identification at the genus level; and a score <1.7 does not enable any identification.

Phylogenetic analysis and classifications
The 16S rRNA gene was sequenced as previously described [14]. The 16S rRNA gene sequence was blasted to the NCBI database for species identification. The strain is considered as a new species if the percentage of similarity is <98.7%. For
phylogenetic classification, homologous sequences from type strains found on the List of Prokaryotic Names with Standing in Nomenclature (LPSN) website (http://www.bacterio.net/) were downloaded from the NCBI database. The phylogenetic tree was constructed using a neighbour-joining method and the maximum composite likelihood substitution model with 1000 bootstraps on MEGA6 (Molecular Evolutionary Genetics Analysis) software [15].

Phenotypic properties and biochemical characterization

Growth of the strain was tested under aerobic conditions with or without 5% CO2 including anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems, respectively (bioMérieux) with different temperatures (25°C, 30°C, 37°C, 42°C and 45°C). Optimal salt concentration was determined by growing the strain at 0%, 0.5%, 2%, 7% and 10% NaCl. Gram staining, motility and sporulation were observed respectively (bioMérieux) with different temperatures (25°C, 37°C, 42°C and 45°C). Optimal salt concentration was determined at 8.04%. The 976 328-paired reads were trimmed. The assembly of the three runs leaded to 3 392 578 paired end reads. SPAdes assembler was used and assembled in to 24 scaffolds.

Genome annotation and comparisons

Open reading frames were predicted using PRODIGAL (http://prodigalornl.gov/) with default parameters. The predicted open reading frames were excluded if they spanned a sequencing gap region. The predicted protein sequences were searched against the GenBank [17] and Clusters of Orthologous Groups database (COGs) databases using BLASTP. The tRNAscanSE tool [18] and RNAmer [19] were used to find tRNA genes and ribosomal RNAs, respectively. Signal peptides and transmembrane helices were predicted by SIGNALP [20] and TMHMM [21]. Furthermore, mobile genetic elements were predicted using PHAST [22] and RAST server [23]. The resistance analysis was identified with the ARG-ANNOT database [24]. Analysis of the presence of polyketide synthases/non-ribosomal peptide synthetases and bacteriocins was identified as previously described [25]. Genomic islands were predicted by ISLANDVIEWER [26]. The genome sequences of available species in the genus Leucobacter were downloaded from NCBI. Average nucleotide identity of draft genomes within the Leucobacter genus was calculated by pairwise comparisons using JSpeciesWS [27]. Moreover, proteome comparisons were performed by rmg-biotools [28] with minimum query coverage of 50% and minimum identity of 50%.

Results

Phylogenetic analysis

After isolation of strain 122RC15, identification was performed by MALDI-TOF analysis. However, MALDI-TOF-MS was unable to identify the strain 122RC15 because its spectrum was not part of the database. BLAST results for 16S rRNA gene sequences showed 98.3% similarity with Leucobacter kyeonggiensis strain F3-P9 (GenBank Accession no. LN849775). The 16S rRNA sequence was deposited in NCBI under GenBank Accession no. LN849775.

Phenotypic analysis

Strain 122RC15 was a Gram-positive rod, non-motile, non-spore-forming (Table 1, Fig. 2), and with mean diameter of 0.3 μm (range 0.3–0.4 μm) and mean length of 0.9 μm (range 0.8–1.0 μm). Colonies were yellow on chocolate agar and
approximately 1 mm in diameter. Strain was able to grow under aerobic conditions with or without 5% CO₂ or microaerophilic conditions between 25 and 45°C after 24 h on Columbia agar with 5% sheep’s blood, chocolate agar and Müller–Hinton agar. No growth occurred under anaerobic conditions. Strain showed catalase-positive but oxidase-negative activities. The strain was able to grow between 0% and 10% NaCl (weight/volume).

Biochemical characterization
For API ZYM, positive reactions were observed for alkaline phosphatase, esterase, esterase-lipase, leucine aminopeptidase, valine arylamidase, cysteine arylamidase, acid phosphatase, and naphthol-AS-Bl-phosphohydrolase. Negative reactions were addressed for lipase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase.

### TABLE 1. Classification and general features of Leucobacter massiliensis strain 122RC15

| Property                  | Term                                      |
|---------------------------|-------------------------------------------|
| Current classification    | Domain: Bacteria                         |
|                           | Phylum: Actinobacteria                    |
|                           | Class: Actinobacteria                     |
|                           | Order: Micrococcales                      |
|                           | Family: Microbacteriaceae                 |
|                           | Genus: Leucobacter                        |
|                           | Species: Leucobacter massiliensis         |
|                           | Strain: 122RC15                           |
| Gram stain                | Gram-positive bacillus                    |
| Cell shape                | Irregular rod-shaped                      |
| Morality                  | Non-motile                                |
| Sporulation               | Non-endospore forming                     |
| Salinity                  | 0%–10% NaCl (weight/volume)              |
| Oxygen requirement        | Aerobic and microaerophilic               |
| Temperature range         | 25 to 45°C                                |
| Optimum temperature       | 37°C                                      |
| Habitat                   | Human pharyngeal                          |
| Isolation                 | Pharynx                                   |

**FIG. 1.** Phylogenetic tree showing relationship of *Leucobacter massiliensis* strain 122RC15 (circle remark) to most closely related species and other representative members of genus *Leucobacter*. The GenBank Accession numbers for 16S rRNA genes are in parenthesis. Tree was constructed using neighbour-joining method and the maximum composite likelihood substitution model with 1000 bootstraps.
N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using an API 50 CH strip (bioMérieux), negative reactions were observed for the fermentation of glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, ribose, D-xyllose, L-xyllose, D-adenitol, methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-xylopyranoside, methyl-α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin, salcin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, amydon, glycogen, xyitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium-5-keto-gluconate. The major fatty acids of strain 122RC15 were branched structures: 15:0 ante-iso (40%); 17:0 ante-iso (38%); 16:0 iso (17%). Moreover, no unsaturated fatty acids were detected. “Leucobacter massiliensis” strain 122RC15 was susceptible to penicillin, amoxicillin, amoxicillin/clavulanic acid, ticarcillin, ceftriaxone, imipenem, gentamicin, kanamycin, trimethoprim/sulfamethoxazole, erythromycin, doxycycline, vancomycin, ciprofloxacin and rifampicin, but resistant to teicoplanin, metronidazole, tobramycin, fosfomycin, nitrofurantoin, sulfamethoxazole and colistin.

Genome description and comparisons

The genome is 3 136 406 bp long with 70.96% G+C content (Table 2). It is composed of 24 scaffolds (composed of 24 contigs). Of the 2846 predicted genes, 2797 were protein-coding genes and 49 were RNAs (two genes are SS rRNA, one gene is 16S rRNA, one gene is 23S rRNA, 45 genes are tRNA genes). A total of 2100 genes (75.08%) were assigned as putative function (by COGs or by NR blast). In all 113 genes were identified as ORFans (4.04%). The remaining 508 genes were annotated as hypothetical proteins (18.16%). Mobilome, virulence, and toxin-associated or antitoxin-associated genes were found in the genome at 48.73%, 20.49% and 2.82%, respectively. Genes associated with polyketide synthases or non-ribosomal peptide synthetases were found at 0.46%. None of genes were associated with antibiotic resistance. However, there are 12 genomic islands and 311 genes predicted in this strain (Fig. 3). The size of the genomic islands varied from 4442 bp to 102 553 bp. Most genomic islands 196 genes (64.95%), had unknown protein function. Ninety-five strain-specific genes such as putative internalin protein, sortase A, LPXTG-specific, ABC-type Fe3+-siderophore transport system were found in genomic islands. The distribution of genes into COGs functional categories for strain 122RC15 is presented in Table 3 and Fig. 4. The genome was deposited into GenBank under accession no. MWZD00000000. Genomes of available Leucobacter

![FIG. 2. (a) Gram staining of Leucobacter massiliensis strain 122RC15. (b) Transmission electron microscopy of Leucobacter massiliensis strain 122RC15 using TechnaiG2 Cryo device (FEI Company) at an operating voltage of 200 keV. Scale bar: 200 nm.](image-url)

### TABLE 2. Nucleotide content and gene count levels of the genome of strain 122RC15

| Attribute                        | Value  | % of total |
|----------------------------------|--------|------------|
| Genome size (bp)                 | 3 136 406 | 100        |
| DNA coding (bp)                  | 2 840 491 | 90.57      |
| DNA G+C (bp)                     | 2 225 715 | 70.96      |
| Total genes                      | 2846   | 100        |
| Protein coding genes             | 2797   | 98.28      |
| RNA genes                        | 49     | 1.72       |
| Genes with function prediction   | 2100   | 75.08      |
| Genes assigned to Clusters of    | 1832   | 65.50      |
| Orthologous Groups database      |        |            |
| Genes with signal peptides       | 327    | 11.69      |
| Genes with transmembrane helices | 697    | 24.92      |
| Genes associated with resistant genes | 0     | 0          |
| Genes associated with polyketide synthases | 13 | 0.46      |
| Genes associated with non-ribosomal peptide synthetases | | |
| Genes associated with bacteriocins | 24    | 0.86       |
| Genes associated with mobilome   | 1363   | 48.73      |
| Genes associated with virulence  | 573    | 20.49      |
| Genes associated with toxin/antitoxin | 79   | 2.82       |
| Genes associated with Pfam-A domains | 2572  | 90         |

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species were compared as shown in Table 4. Average nucleotide identity between *Leucobacter* genomes showed that strain 122RC15 has similarity with *Leucobacter chironomi* DSM 19883 at 79.42% identity (Table 5).

Moreover, all-against-all BLAST comparison of multiple genomes was performed using coding sequences as shown in Fig. 5. Strain 122RC15 has the highest protein similarity 43.2% with *L. chironomi* DSM 19883. Homology among *Leucobacter* proteomes ranges from 29.5% to 85.8%.

Several species of *Leucobacter* have been found to be resistant to chromium [29–31]. However, strain 122RC15 lacks the chromate transporter (*Chr*) gene. Moreover, salt-tolerance-related genes, such as the ABC-type proline/glycine betaine transporters, are absent.

**FIG. 3.** Genomic islands of *Leucobacter massiliensis* strain 122RC15. Orange and blue lines denote genomic islands predicted by SIGI-HMM and IslandPath/DIMOB, respectively. Red line denotes the genomic locations of all predicted islands.

**FIG. 4.** Graphical circular map of *Leucobacter massiliensis* strain 122RC15 chromosome. From outside to centre: outer two circles show open reading frame oriented forward (coloured by the Clusters of Orthologous Groups database categories) and backwards (coloured by Clusters of Orthologous Groups database categories), respectively. Third circle marks tRNA genes (green). Fourth circle shows G+C% content plot. Innermost circle shows GC skew; purple indicates negative values and olive indicates positive values.

**TABLE 3.** Number of genes associated with the general COGs functional categories

| Code | Value | % value | Description |
|------|-------|---------|-------------|
| J    | 168   | 6.01    | Translation |
| A    | 1     | 0.04    | RNA processing and modification |
| K    | 139   | 4.97    | Transcription |
| L    | 91    | 3.25    | Replication, recombination and repair |
| B    | 2     | 0.07    | Chromatin structure and dynamics |
| D    | 23    | 0.82    | Cell cycle control, mitosis and meiosis |
| Y    | 0     | 0.00    | Nuclear structure |
| V    | 54    | 1.93    | Defence mechanisms |
| T    | 62    | 2.22    | Signal transduction mechanisms |
| M    | 78    | 2.79    | Cell wall/membrane biogenesis |
| N    | 13    | 0.46    | Cell motility |
| Z    | 0     | 0.00    | Cytoskeleton |
| W    | 1     | 0.04    | Extracellular structures |
| U    | 24    | 0.86    | Intracellular trafficking and secretion |
| O    | 73    | 2.61    | Post-translational modification, protein turnover, chaperones |
| X    | 10    | 0.36    | Methylomix propligas, transposition |
| C    | 111   | 3.97    | Energy production and conversion |
| G    | 160   | 5.72    | Carbohydrate transport and metabolism |
| E    | 334   | 11.94   | Amino acid transport and metabolism |
| F    | 69    | 2.47    | Nucleotide transport and metabolism |
| H    | 93    | 3.32    | Coenzyme transport and metabolism |
| I    | 97    | 3.47    | Lipid transport and metabolism |
| P    | 151   | 5.40    | Inorganic ion transport and metabolism |
| Q    | 57    | 2.04    | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 198   | 7.08    | General function prediction only |
| S    | 76    | 2.72    | Unknown function |
| X    | 712   | 25.46   | Not in COGs |

**TABLE 4.** List of *Leucobacter* genomes and their GenBank Accession number

| Organism/Name | Ref seq accession no. | Size (Mb) | GC% |
|---------------|-----------------------|-----------|-----|
| *Leucobacter* sp. PH1c | NZ_AYPV00000000.0 | 3.12 | 71.30 |
| *Leucobacter* sp. Ag1 | NZ_LAYO00000000.0 | 3.54 | 70.30 |
| *Leucobacter* sp. UCD-THU | NZ_ATXL00000000.0 | 3.32 | 70.30 |
| *Leucobacter* sp. G161 | NZ_LOHP00000000.0 | 3.55 | 65.30 |
| *Leucobacter* musarum subsp. musarum CBX152 | NZ_JHMX00000000.0 | 3.44 | 66.80 |
| *Leucobacter* musarum subsp. japonicus CBX130 | NZ_JHMX00000000.0 | 3.59 | 66.80 |
| *Leucobacter* celor subsp. astriofaciens CBX151 | NZ_JHMX00000000.0 | 4.14 | 69.10 |
| *Leucobacter* chironomi DSM 19883 | NZ_ATXU00000000.0 | 2.96 | 69.90 |
| *Leucobacter* salicis M1-8 | NZ_AOCC00000000.0 | 3.19 | 64.50 |
| *Leucobacter* chironomi DSM 19883 | NZ_AGCH00000000.0 | 3.37 | 68.40 |
| *Leucobacter* chironomi DSM 19883 | NZ_LDRX00000000.0 | 2.79 | 70.80 |
| *Leucobacter* chironomi DSM 22788 | NZ_FNKX00000000.0 | 3.22 | 70.30 |
| *Leucobacter* komagatae VKM ST2845 | NZ_KSCL00000000.0 | 3.67 | 65.30 |
| *Leucobacter* massiliensis 122RC15 | MWZD00000000 | 3.14 | 70.96 |

Abbreviation: COGs, Cluster of Orthologous Groups database.
transport system, were found in this 122RC15 genome, indicating salt-tolerance properties of “L. massiliensis”. A total of 551 specific genes, including six genes of putative internalin, multidrug-efflux transporter and sarcosine operon, were found in this strain.

Conclusion

On the basis of phenotypic, phylogenetic and genomic analyses (taxonogenomics), we formally propose the creation of “Leucobacter massiliensis” sp. nov., which contains the strain 122RC15. This bacterium has been isolated from the pharynx of a healthy 76-year-old Algerian woman after traveling to the 2014 Hajj.

Description of Leucobacter massiliensis sp. nov.

“Leucobacter massiliensis” name comes from Massilia, the ancient Roman name for Marseille, France, where the type strain was isolated. The strain was aerobic, Gram-positive, rod-shaped, non-spore-forming, non-motile. Growth was achieved aerobically between 25 and 45°C after 24 h. Catalase was positive but oxidase was negative. The genome is 3 136 406 bp long and G+C content is 70.3%. The 16S rRNA gene sequence and whole-genome shotgun sequence of L. massiliensis strain 122RC15 are deposited in GenBank under Accession numbers LN849775 and MWZD00000000, respectively. The type strain 122RC15 (= DSM 29913 = CSUR P1430) was isolated from the pharynx of a healthy 76-year-old Algerian woman after traveling to the 2014 Hajj.

### Table 5. Pairwise comparison of Leucobacter massiliensis with 13 other species using JSPECIESWS (measures the average nucleotide identity (ANI) based on BLAST+ (ANIb))

| Genome no. | Organism name                        | 1   | 2      | 3      | 4      | 5      | 6      | 7      | 8      | 9      | 10     | 11     | 12     | 13     | 14     |
|------------|-------------------------------------|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1          | Leucobacter saliscius M1-8           | *75.56 | 76.21  | 76.11  | 75.97  | 74.71  | 75.75  | 74.53  | 73.88  | 74.55  | 75.48  | 73.2   | 75.39  | 75.34  |
| 2          | Leucobacter chlorisresistens JG 31   | *78.81 | 78.79  | 78.32  | 76.92  | 78.64  | 80.14  | 74.6   | 80.25  | 94     | 74.29  | 99.96  | 77.88  |
| 3          | Leucobacter sp. UCD-THU              | *87.03 | 78.92  | 77.6   | 80.64  | 74.7   | 77.29  | 78.49  | 74.53  | 78.57  | 79.19  |
| 4          | Leucobacter chlorisresistens DSM 19883 | *79.13 | 77.75  | 80.58  | 77.38  | 75.41  | 77.28  | 79.72  | 75.12  | 78.76  | 79.42  |
| 5          | Leucobacter sp. Ph1c                | *77.67 | 78.93  | 76.87  | 76.51  | 76.93  | 75.86  | 75.4   | 78.41  | 76.44  |
| 6          | Leucobacter sp. Ag1                  | 77.55  | 75.5   | 74.07  | 75.58  | 76.95  | 73.96  | 76.38  | 76.51  |
| 7          | Leucobacter celor subsp. astrophilus CBX151 | *77.21 | 74.14  | 77.55  | 77.71  | 73.84  | 77.76  | 79.35  | 79.35  |
| 8          | Leucobacter musurum subsp. musurum CBX152 | *73.57 | 94.67  | 79.39  | 73.39  | 79.57  | 75.92  |
| 9          | Leucobacter sp. G161                | *73.31 | 74.44  | 79.3   | 74.42  | 74.49  |
| 10         | Leucobacter musurum subsp. japonicus CBX130 | *79.24 | 73.21  | 79.35  | 75.88  |
| 11         | Leucobacter chlorisresistens N5354  | *74.64  | 94.02  | 78.04  |
| 12         | Leucobacter komagater VKM ST2845    | *73.95 | 73.79  |
| 13         | Leucobacter chlorisresistens DSM 22788 | 78.1   | 78.1   |
| 14         | Leucobacter massiliensis 122RC15    | 78.1   | 78.1   |

**FIG. 5.** A BLAST matrix of an all-against-all protein comparison of 14 Leucobacter genomes. Percentages of proteome comparisons were calculated by core genes divided by pan genes between genomes.
Conflict of interest

None to declare.

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