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 122RC15^T 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 2797 protein-coding genes and 49 RNA genes.

Keywords: Culturomics, genome, Hajj, *Leucobacter massiliensis*, new species, taxonogenomics
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Introduction

"*Leucobacter massiliensis*" strain 122RC15^T (= DSM 29913= CSUR P1430) is the type strain of *L. massiliensis* sp. nov. This bacterium was isolated from the pharynx of a 76-year-old woman originating from Algeria and living in Marseille after travelling from the 2014 Hajj [1]. This pilgrim had respiratory tract symptoms including cough, sore throat and loss of voice but without antibiotic usage during the Hajj. The genus *Leucobacter* was first designed by Takeuchi et al. [2] and formerly included 25 validly described species. Members of *Leucobacter* have been isolated from several sources including waste-water [3–7], soil environments [8,9], air [10], foods [11] and nematodes [12]. However, there are no known human pathogens among the genus *Leucobacter*.

"*L. massiliensis*" is an aerobic, Gram-positive bacillus. With current knowledge, this strain 122RC15 is the first species of *Leucobacter* genus isolated from human pharynx. Here, we describe the main characteristics of strain 122RC15 with a phenotypic and phylogenetic analysis and the complete genome description.

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% CO_2 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 pattern 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 $\geq$2 with a validly published species enabled identification at the species; a score $\geq$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

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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% CO_2 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 using a light microscope (DM1000; Leica Microsystems, Nanterre, France). Electron microscopy of "*L. massiliensis*" was performed using a TechnaiG2 Cryo (FEI Company, Limeil-Brevannes, France) at an operating voltage of 200 keV.

Biochemical assays were characterized using API ZYM and API 50 CH (bioMérieux) gallery systems. Cellular fatty acid methyl ester analysis was performed by gas chromatography/mass spectrometry as previously described [16].

Genome sequencing and assembly

Genomic DNA of "*L. massiliensis*" was sequenced using the MiSeq Technology (Illumina, San Diego, CA, USA) with the pair end and mate-pair strategies. First, automated cluster generation and sequencing runs were performed in a single 39-h run at a 2 × 250-bp read length. Total information of 7.3 Gb was obtained from an 800 K/mm^2 cluster density, with a cluster passing quality control filters of 92.2% (14 034 000 passing filter clusters). Within this run, the index representation for "*L. massiliensis*" was 10.25%. The 1 438 190 paired reads were trimmed. Moreover, two mate-pair libraries were performed in a single 39-h run in a 2 × 151-bp read length. The first library was loaded twice in the same run where total information of 6.1 Gb was obtained from a 653 K/mm^2 cluster density with a cluster passing quality control filters of 96.1% (12 031 000 passing filter paired reads). Within this run, the index representation for "*L. massiliensis*" was 4.29%. The 978 060 paired reads were trimmed then assembled. The second library was loaded once and 6.5 Gb of total information was obtained from a 696 K/mm^2 cluster density with a cluster passing quality control filters of 95.6% (12 863 000 passing filter paired reads). Within this run, the index representation for "*L. massiliensis*" 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://prodigal.ornl.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 RNAmmer [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 resistome 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 CMG-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. NR132679.1), which was confirmed by phylogenetic tree construction (Fig. 1). Hence, we propose the creation of a new species, which putatively classifies it as a member of the *Leucobacter* genus within the *Microbacteriaceae* family in the *Actinobacteria* phylum (Table 1). 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 0.35 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% CO_2 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 arylamidase, valine arylamidase, cysteine arylamidase, acid phosphatase, and naphthol-AS-BI-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* 122RC15
Gram stain	Gram-positive bacillus
Cell shape	Irregular rod-shaped
Motility	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

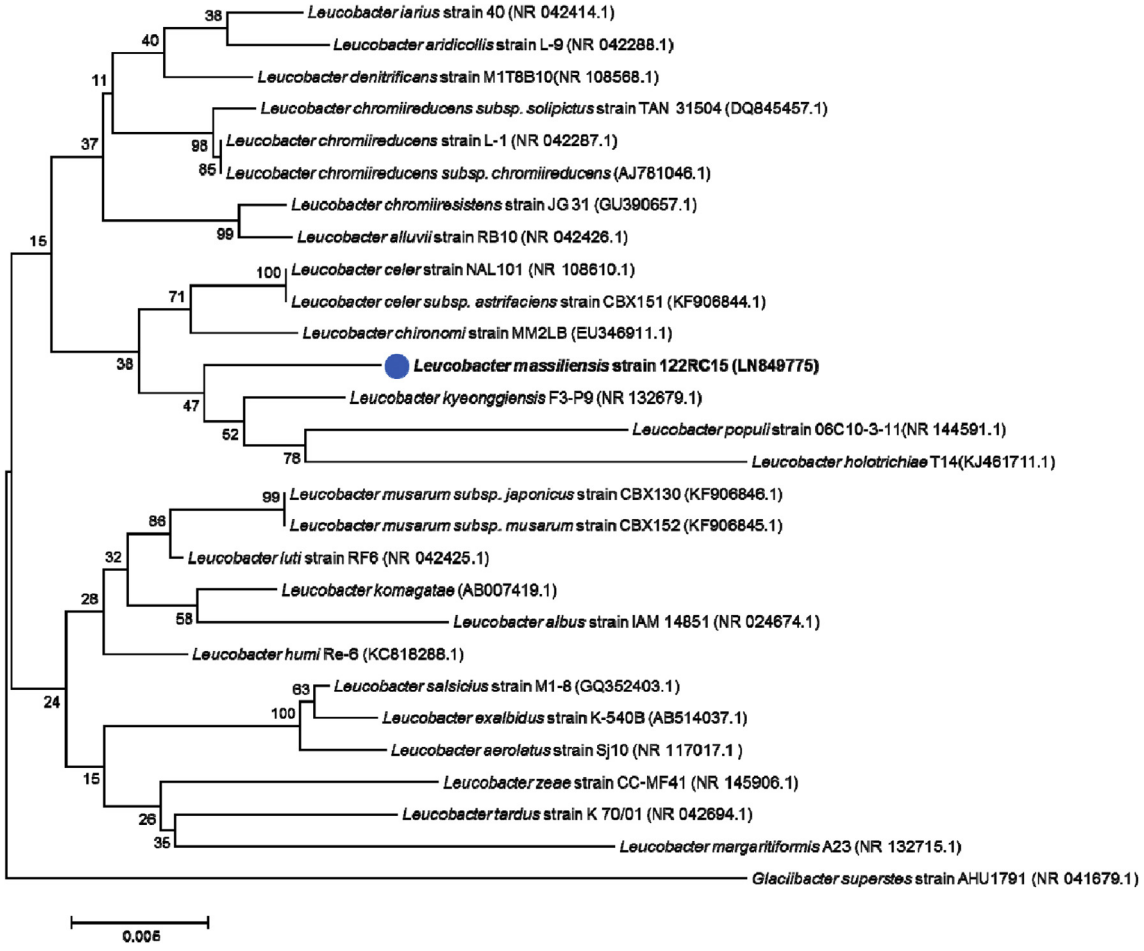

FIG. 1. Phylogenetic tree showing relationship of *Leucobacter massiliensis* strain 122RC15 (circle remark) to most closely related species and other representative members of genus *Leucobacter*. The GenBank Accession numbers for 16S rRNA genes are in parenthesis. Tree was constructed using neighbour-joining method and the maximum composite likelihood substitution model with 1000 bootstraps.

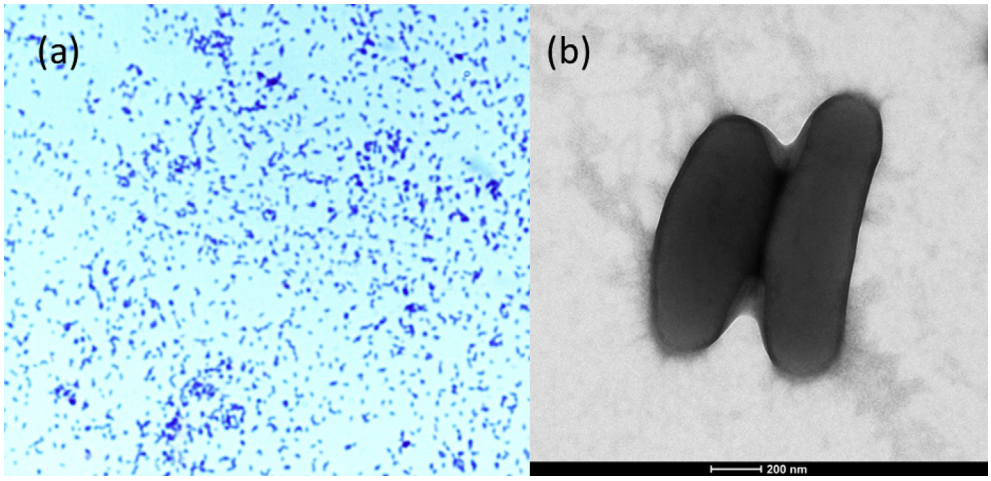

FIG. 2. (a) Gram staining of *Leucobacter massiliensis* strain 122RC15. (b) Transmission electron microscopy of *Leucobacter massiliensis* strain 122RC15 using TechnaiG2 Cryo device (FEI Company) at an operating voltage of 200 keV. Scale bar: 200 nm.

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-xylose, L-xylose, D-adonitol, 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, salicin, D-cellobiose, D-maltose, D-lactose, D-mellibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, amidon, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium-5 ketogluconate. 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, nitrofuratoin, 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 5S 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 Fe^{3+}-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*

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 Orthologous Groups database	1832	65.50
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 or non-ribosomal peptide synthetases	13	0.46
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

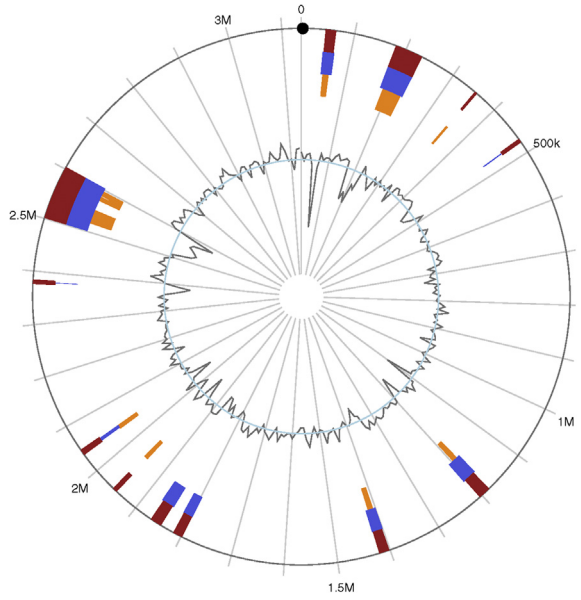

FIG. 3. Genomic islands of *Leucobacter massiliensis* strain 122RC15. Orange and blue lines denote genomic islands predicted by SIGI-HMM and IslandPath/DIMOB, respectively. Red line denotes the genomic locations of all predicted islands.

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	Mobilome: prophages, transposon
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
–	712	25.46	Not in COGs

Abbreviation: COGs, Cluster of Orthologous Groups database.

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).

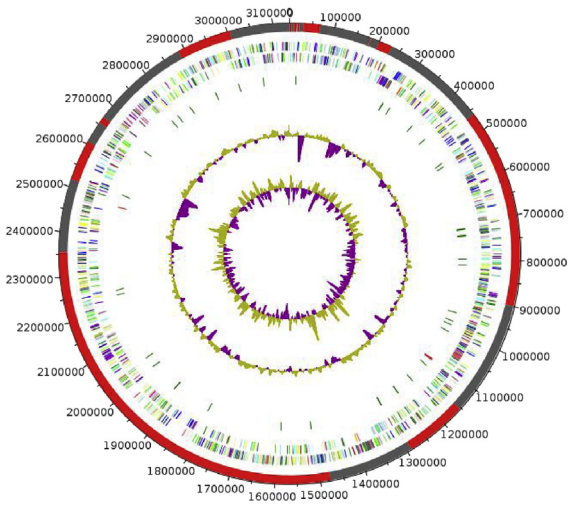

FIG. 4. Graphical circular map of *Leucobacter massiliensis* strain 122RC15 chromosome. From outside to centre: outer two circles show open reading frame oriented forward (coloured by the Clusters of Orthologous Groups database categories) and backwards (coloured by Clusters of Orthologous Groups database 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 4. List of *Leucobacter* genomes and their GenBank Accession number

Organism/Name	Ref seq accession no.	Size (Mb)	GC%
Leucobacter sp. PH1c	NZ_AYMV00000000.1	3.12	71.30
Leucobacter sp. Ag1	NZ_LAYO00000000.1	3.54	70.30
Leucobacter sp. UCD-THU	NZ_APJM00000000.1	3.32	70.30
Leucobacter sp. G161	NZ_LOHP00000000.1	3.55	65.30
Leucobacter musarum subsp. *musarum* CBX152	NZ_JHBW00000000.1	3.44	66.80
Leucobacter musarum subsp. *japonicus* CBX130	NZ_JHBX00000000.1	3.59	66.80
Leucobacter celer subsp. *astrifaciens* CBX151	NZ_JHEI00000000.1	4.14	69.10
Leucobacter chironomi DSM 19883	NZ_ATXU00000000.1	2.96	69.90
Leucobacter salsicius M1-8	NZ_AOCN00000000.1	3.19	64.50
Leucobacter chromiiresistens JG 31	NZ_AGCW00000000.1	3.37	68.40
Leucobacter chromiiresistens NS354	NZ_LDRK00000000.1	2.79	70.80
Leucobacter chromiiresistens DSM 22788	NZ_FNKB00000000.1	3.22	70.30
Leucobacter komagatae VKM ST2845	NZ_JXSQ00000000.1	3.67	65.30
Leucobacter massiliensis 122RC15	MWZD00000000	3.14	70.96

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

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 salsicius* 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 chromiiresistens* JG 31		*	78.81	78.79	78.32	76.92	78.64	80.14	74.6	80.25	94	74.29	99.98	77.88
3	*Leucobacter* sp. UCD-THU			*	87.03	78.92	77.6	80.64	77.4	74.75	77.29	78.49	74.53	78.57	79.19
4	*Leucobacter chironomi* DSM 19883				*	79.13	77.75	80.58	77.38	75.41	77.28	78.72	75.12	78.76	79.42
5	*Leucobacter* sp. PH1c					*	77.67	78.93	76.87	75.31	76.93	78.26	75.4	78.41	78.44
6	*Leucobacter* sp. Ag1						*	77	75.5	74.07	75.58	76.95	73.96	76.58	76.51
7	*Leucobacter celer* subsp. *astrifaciens* CBX151							*	77.21	74.14	77.55	77.71	73.84	77.76	79.35
8	*Leucobacter musarum* subsp. *musarum* 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 musarum* subsp. *japonicus* CBX130										*	79.24	73.21	79.35	75.88
11	*Leucobacter chromiiresistens* NS354											*	74.64	94.02	78.04
12	*Leucobacter komagatae* VKM ST2845												*	73.95	73.79
13	*Leucobacter chromiiresistens* DSM 22788													*	78.1
14	*Leucobacter massiliensis* 122RC15														

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.

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 pilgrim returning from 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 travelling to the 2014 Hajj.

Conflict of interest

None to declare.

Acknowledgement

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