Non contiguous-finished genome sequence and description of *Enterobacter massiliensis* sp. nov.

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*Enterobacter massiliensis* strain JC163ᵀ is the type strain of *E. massiliensis* sp. nov., a new species within the genus *Enterobacter*. This strain, whose genome is described here, was isolated from the fecal flora of a healthy Senegalese patient. *E. massiliensis* is an aerobic rod. Here we describe the features of this organism, together with the complete genome sequence and annotation. The 4,922,247 bp long genome (1 chromosome but no plasmid) exhibits a G+C content of 55.1% and contains 4,644 protein-coding and 80 RNA genes, including 5 rRNA genes.

Introduction

*Enterobacter massiliensis* strain JC163ᵀ (= CSUR P161 = DSM 26120) is the type strain of *E. massiliensis* sp. nov. This bacterium is a Gram-negative, aerobic, flagellate, indole-positive bacillus that was isolated from the feces of a healthy Senegalese patient in a study aiming at cultivating all bacterial species in human feces [1]. The current classification of prokaryotes, known as polyphasic taxonomy, relies on a combination of phenotypic and genotypic characteristics [2]. However, as more than 3,000 bacterial genomes have been sequenced [3] and the cost of genomic sequencing is decreasing, we recently proposed to integrate genomic information in the description of new bacterial species [4-15].

Here we present a summary classification and a set of features for *E. massiliensis* sp. nov. strain JC163ᵀ (= CSUR P161 = DSM 26120), together with the description of the complete genomic sequencing and annotation. These characteristics support the circumscription of the species *E. massiliensis*. The genus *Enterobacter* (Hormaeche and Edwards, 1960) was created in 1960 [16]. To date, this genus is comprised of 25 species [17-35] and 2 subspecies. Members of the genus were isolated mostly from the environment, in particular from plants and fruits, but are also frequently isolated from humans, notably in health-care associated infections, causing bacteremia, pneumonia or urinary tract infections [36]. In addition, many *Enterobacter* spp. were isolated from the normal fecal flora.

Classification and features

A stool sample was collected from a healthy 16-year-old male Senegalese volunteer patient living in Dielmo (rural village in the Guinean-Sudanian zone in Senegal), who was included in a research protocol. Written assent was obtained from this individual. No written consent was needed from his guardians for this study because he was older than 15 years old (in accordance with the previous project approved by the Ministry of Health of Senegal and the assembled village population and as published elsewhere [37]).

Both this study and the assent procedure were approved by the National Ethics Committee of Senegal (CNERS) and the Ethics Committee of the Institut Fédératif de Recherche IFR48, Faculty of Medicine, Marseille, France (agreement numbers 09-022 and 11-017). Several other new bacterial species were isolated from this specimen using various culture conditions, including the recently described *Alistipes senegalensis*, *Alistipes timonensis*, *Anaerococcus senegalensis*, *Bacillus timonensis*, *Clostridium senegalense*, *Peptoniphilus timonensis*, *Paenibacillus senegalensis*, *Herbaspirillum massiliense*, *Kurthia massiliensis*,...
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Brevibacterium senegalense, Aeromicrobium massiliense and Cellulomonas massiliensis [4-15].

The fecal specimen was preserved at -80°C after collection and sent to Marseille. Strain JC163T (Table 1) was isolated in April 2011 by aerobic cultivation on Brain-Heart Infusion (BHI) agar at 37°C after preincubation of the stool specimen with lytic E. coli T1 and T4 phages [1,50]. This strain exhibited a nucleotide sequence similarity with Enterobacter species ranging from 95.74% with E. pyrinus (Chung et al., 1993) to 97.33% with E. cloacae subsp. cloacae (Jordan, 1980) (Figure 1). This latter value was lower than the 98.7% 16S rRNA gene sequence threshold recommended by Stackebrandt and Ebers to delineate a new species without carrying out DNA-DNA hybridization [51].

Table 1. Classification and general features of Enterobacter massiliensis strain JC163T

| MIGS ID | Property            | Term                      | Evidence codea |
|---------|---------------------|---------------------------|----------------|
|         | Domain: Bacteria    | TAS [38]                  |                |
|         | Phylum Proteobacteria| TAS [39]                  |                |
|         | Class Gammaproteobacteria | TAS [40,41] |                |
| Current classification | Order “Enterobacterales” | TAS [42] |                |
|         | Family Enterobacteriaceae | TAS [43-45] |                |
|         | Genus Enterobacter | TAS [16,44,46-48] |                |
|         | Species Enterobacter massiliensis | IDA |                |
|         | Type strain: JC163T | IDA |                |
|         | Gram stain          | Negative                  | IDA            |
|         | Cell shape          | Rod                       | IDA            |
|         | Motility            | Motile                    | IDA            |
|         | Sporulation         | Nonsporulating            | IDA            |
|         | Temperature range   | Mesophile                 | IDA            |
|         | Optimum temperature | 37°C                      | IDA            |
| MIGS-6.3 | Salinity           | Unknown                   | IDA            |
| MIGS-22 | Oxygen requirement  | Aerobic                   | IDA            |
|         | Carbon source       | Unknown                   | NAS            |
|         | Energy source       | Unknown                   | NAS            |
| MIGS-6 | Habitat             | Human gut                 | IDA            |
| MIGS-15 | Biotic relationship | Free living              | IDA            |
|         | Pathogenicity       | Unknown                   | IDA            |
|         | Biosafety level     | 2                         | IDA            |
| MIGS-14 | Isolation          | Human feces               | IDA            |
| MIGS-4 | Geographic location | Senegal                   | IDA            |
| MIGS-5 | Sample collection time | September 2010 | IDA            |
| MIGS-4.1 | Latitude           | 13.7167                   | IDA            |
| MIGS-4.1 | Longitude          | -16.4167                  | IDA            |
| MIGS-4.3 | Depth              | Surface                   | IDA            |
| MIGS-4.4 | Altitude           | 51 m above sea level      | IDA            |

Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [49]. If the evidence is IDA, then the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.
Figure 1. Phylogenetic tree highlighting the position of \textit{Enterobacter massiliensis} strain JC163 relative to other type strains within the \textit{Enterobacter} genus. Members of phylogenetically closely related genera were also included. GenBank accession numbers are indicated in parentheses. Sequences were aligned using CLUSTALW, and phylogenetic inferences obtained using the maximum-likelihood method within the MEGA software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. The scale bar represents a 0.2\% nucleotide sequence divergence.
Different growth temperatures (25, 30, 37, 45°C) were tested; growth occurred between 25 and 45°C, optimal growth was observed between 30 and 37°C. Colonies were convex, opaque, light-cream colored and circular with regular margins and with a diameter of 2 mm on BHI agar. Growth of the strain was tested under anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems, respectively (BioMerieux), and under aerobic conditions, with or without 5% CO₂. Optimal growth was obtained under aerobic conditions in the presence of 5% CO₂. Weak growth was observed under microaerophilic condition. No growth was obtained anaerobically. A motility test was positive. Cells grown on agar are Gram-negative rods (Figure 2) and have a mean diameter of 1.02 µm and a mean length of 1.90 µm and have several polar flagella (Figure 3).

Figure 2. Gram staining of *E. massiliensis* strain JC163³

Figure 3. Transmission electron microscopy of *E. massiliensis* strain JC163³, using a Morgani 268D (Philips) at an operating voltage of 60kV. The scale bar represents 500 nm.
Strain JC163<sup>T</sup> exhibited catalase activity but not oxidase activity. Using the API 20E system, positive reactions were obtained for indole production, β-galactosidase and glucose, mannitol, sorbitol and rhamnose fermentation. *E. massiliensis* is susceptible to ticarcillin, imipenem, trimethoprim/sulfamthoxazole, gentamicin, amikacin, and colimycin but resistant to fosfomycin and nitrofurantoin. By comparison with *E. arachidis*, its phylogenetically-closest neighbor, *E. massiliensis* differed in arginine dihydrolase, ornithine decarboxylase, citrate and succinate fermentation [19].

Matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) MS protein analysis was carried out as previously described [52]. Briefly, a pipette tip was used to pick one isolated bacterial colony from a culture agar plate and spread it as a thin film on a MTP 384 MALDI-TOF target plate (Bruker Daltonics, Germany). Twelve distinct deposits were done for strain JC163<sup>T</sup> from twelve isolated colonies. Each smear was overlaid with 2 µL of matrix solution (saturated solution of alpha-cyano-4-hydroxycinnamic acid) in 50% acetonitrile, 2.5% tri-fluoracetic acid, and allowed to dry for five minutes. Measurements were performed with a Microflex spectrometer (Bruker). Spectra were recorded in the positive linear mode for the mass range of 2,000 to 20,000 Da (parameter settings: ion source 1 (ISI), 20kV; IS2, 18.5 kV; lens, 7 kV). A spectrum was obtained after 675 shots at a variable laser power. The time of acquisition was between 30 seconds and 1 minute per spot. The twelve JC163<sup>T</sup> spectra were imported into the MALDI BioTyper software (version 2.0, Bruker) and analyzed by standard pattern matching (with default parameter settings) against the main spectra of 3,769 bacteria, including spectra from 34 validly published *Enterobacter* species that were used as reference data in the BioTyper database (updated March 15th, 2012). The method of identification includes the m/z from 3,000 to 15,000 Da. For every spectrum, a maximum of 100
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peaks were taken into account and compared with the spectra in database. A score enabled the presumptive identification and discrimination of the tested species from those in a database: a score ≥ 2 with a validly published species enabled the identification at the species level; a score ≥ 1.7 but < 2 enabled the identification at the genus level; and a score < 1.7 did not enable any identification. For strain JC163T, the score obtained was 1.4, suggesting that our isolate was not a member of a known species. We incremented our database with the spectrum from strain JC163T (Figure 4). In addition, the gel view allows the highlighting of spectra differences with other of Enterobacteriaceae family members (Figure 5).

Figure 5. Gel view comparing Enterobacter massiliensis JC163 spectra with 23 other members into Enterobacteriaceae family. The Gel View displays the raw spectra of all loaded spectrum files arranged in a pseudo-gel like look. The x-axis records the m/z value. The left y-axis displays the running spectrum number originating from subsequent spectra loading. The peak intensity is expressed by a Gray scale scheme code. The color bar and the right y-axis indicate the relation between the color a peak is displayed with and the peak intensity in arbitrary units.

Genome sequencing information

Genome project history

The organism was selected for sequencing on the basis of its phylogenetic position and 16S rRNA similarity to other members of the genus Enterobacter, and is part of a study of the human digestive flora aiming at isolating all bacterial species within human feces. It was the 10th genome of an Enterobacter species (including genomes from 5 validly published species) and the first genome of E. massiliensis sp. nov. The genome sequence deposited in GenBank under accession number CAEO000000000 consists of 224 contigs. Table 2 shows the project information and its association with MIGS version 2.0 compliance [53].

Growth conditions and DNA isolation

E. massiliensis strain JC163T, (= CSUR P161 = DSM 26120) was grown aerobically on BHI agar at 37°C. Four petri dishes were spread and resuspended in 3×100μl of G2 buffer. A first mechanical lysis was performed by glass powder on the Fastprep-24 device (Sample Preparation system, MP Biomedicals, USA) during 2×20 seconds. DNA was then treated with 2.5 μg/μl lysozyme (30 minutes at 37°C) and extracted through the BioRobot EZ 1 Advanced XL (Qiagen). The DNA was then concentrated and purified on a Qiamp kit (Qiagen). The yield and the concentration was measured by the Quant-it Picogreen kit (Invitrogen) on the Genios_Tecan fluorometer at 118 ng/μl.
Table 2. Project information

| MIGS ID | Property          | Term                              |
|---------|-------------------|-----------------------------------|
| MIGS-31 | Finishing quality | High-quality draft                |
| MIGS-28 | Libraries used    | Paired-end 3 Kb library           |
| MIGS-29 | Sequencing platforms | 454 GS FLX Titanium             |
| MIGS-31.2 | Fold coverage       | 13×                              |
| MIGS-30 | Assemblers        | Newbler version 2.5.3             |
| MIGS-32 | Gene calling method | Prodigal                     |
|         | EMBL ID           | CAEO00000000                      |
|         | EMBL Date of Release | November 19, 2012              |
| MIGS-13 | Project relevance | Study of the human gut microbiome|

Genome sequencing and assembly

A 3kb paired-end sequencing strategy (Roche, Meylan, France) was used. Five µg of DNA was mechanically fragmented on the Hydroshear device (Digilab, Holliston, MA, USA) with an enrichment size at 3-4kb. The DNA fragmentation was visualized using an Agilent 2100 BioAnalyzer on a DNA labchip 7500, with an optimal size of 4.648kb. The library was constructed according to the 454_Titanium paired end protocol (Roche). Circularization and nebulization were performed and generated a pattern with an optimal at 437 bp. Following PCR amplification through 15 cycles followed by double size selection, the single stranded paired-end library was then quantified on the Quant-it Ribogreen kit (Invitrogen) on the Genios_Tecan fluorometer at 122pg/µL. The library concentration equivalence was calculated as 5.12E+08 molecules/µL. The library was stored at -20°C until use. The library was clonally amplified with 1 cpb in 4 emPCR reactions with the GS Titanium SV emPCR Kit (Lib-L) v2 (Roche). The yield of the emPCR was 14.45%, in the 5 to 20% range recommended by the Roche procedure.

Approximately 790,000 beads were loaded on ¼ region of a GS Titanium PicoTiterPlate (PTP Kit 70x75, Roche) and pyrosequenced with the GS Titanium Sequencing Kit XLR70 and the GS FLX Titanium sequencer (Roche). The run was performed overnight and then analyzed on the cluster through the gsRunBrowser and Newbler assembler (Roche). A total of 283,817 passed filter wells generated 80.8 Mb with a length average of 284 bp. The passed filter sequences were assembled using Newbler with 90% identity and 40 bp as overlap. The final assembly identified 224 contigs arranged in 11 scaffolds and generated a genome size of 4.92 Mb.

Genome annotation

Open Reading Frames (ORFs) were predicted using Prodigal [54] with default parameters but the predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against the GenBank database [55] and the Clusters of Orthologous Groups (COG) databases using BLASTP. The tRNAscanSE tool [56] was used to find tRNA genes, whereas ribosomal RNAs were found by using RNAmmer [57] and BLASTN against the GenBank database. Signal peptides and numbers of transmembrane helices were predicted using SignalP [58] and TMHMM [59] respectively.

To estimate the mean level of nucleotide sequence similarity at the genome level between *E. massiliensis* strain JC163, *E. aerogenes* strain KCTC 2190 (GenBank accession number CP002824), *E. asburiae* strain LF7a (CP003026), *E. cancerogenus* strain ATCC35316 (ABWM00000000), *E. cloacae* subsp. *cloacae* strain ATCC13047 (CP0001918), *E. cloacae* subsp. *dissolvens* strain SDM (CP003678) and *E. hormaechei* strain ATCC49162 (AFHR00000000), we compared the ORFs only using BLASTN and the following parameters: a query coverage of > 70% and a minimum nucleotide length of 100 bp.
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Figure 6. Graphical circular map of the chromosome. From outside to the center: genes on both the forward and reverse strands, genes on forward strand, genes on reverse strand, genes colored by COG categories, RNA genes (tRNAs and rRNAs) and blast of the genome vs itself.

Genome properties

The genome of *E. massiliensis* sp. nov. strain JC163^T^ is 4,922,247 bp long (1 chromosome but no plasmid) with a 55.1% G+C content (Figure 6 and Table 3). Of the 4,724 predicted genes, 4,644 were protein-coding genes, and 80 were RNAs, including 1 complete rRNA operon, 2 additional 5S rRNAs and 75 tRNAs. A total of 3,181 genes (68.5%) were assigned a putative function. The remaining genes were annotated as hypothetical or unknown proteins. The distribution of genes into COGs functional categories is presented in Table 4. The properties and the statistics of the genome are summarized in Tables 3 and 4.
Comparison with other Enterobacter species genomes

Here, we compared the genome of *E. massiliensis* strain JC163 with those of *E. aerogenes* strain KCTC 2190, *E. asburiae* strain LF7a, *E. cancerogenus* strain ATCC35316, *E. cloacae* subsp. *dissolvens* strain ATCC13047, *E. cloacae* subsp. SDM and *E. hormaechei* strain ATCC49162. The draft genome of *E. massiliensis* is smaller than those of *E. aerogenes*, *E. cloacae* subsp. *cloacae* and *E. cloacae* subsp. *dissolvens* (4.92, 5.28, 5.59 and 4.96Mb, respectively), but larger than those of *E. asburiae*, *E. cancerogenus* and *E. hormaechei* (3.81, 4.60 and 4.80, respectively). *E. massiliensis* has a similar G + C content to *E. cloacae* subsp. *dissolvens* (55.1%) but larger than *E. aerogenes*, *E. asburiae* and *E. cloacae* subsp. *cloacae* (54.8, 53.8 and 54.79%, respectively) and lower than *E. cancerogenus* and *E. hormaechei* (55.8 and 55.2%, respectively). *E. massiliensis* had a greater number of predicted genes than *E. cancerogenus* and *E. cloacae* subsp. *dissolvens* (5,724, 4,642 and 4,646, respectively), but a smaller number than *E. aerogenes*, *E. asburiae*, *E. cloacae* subsp. *cloacae* and *E. hormaechei* (5,021, 4,805, 5,627 and 4,779, respectively).

In addition, *E. massiliensis* shared a mean genome sequence similarity of 84.26% (range 70.05-100%), 83.89% (70.03-100%), 84.36% (70.05-100%), 84.14% (70.00-100%), 84.14% (70.05-100%) and 84.38% (70.24-100%) with *E. aerogenes*, *E. asburiae*, *E. cancerogenus*, *E. cloacae* subsp. *cloacae*, *E. cloacae* subsp. *dissolvens* and *E. hormaechei*, respectively.

Table 3. Nucleotide content and gene count levels of the genome

| Attribute                       | Value   | % of totala |
|---------------------------------|---------|-------------|
| Genome size (bp)                | 4,922,247 |            |
| DNA coding region (bp)          | 4,284,197 | 87         |
| DNA G+C content (bp)            | 2,712,016 | 55.1       |
| Total genes                     | 4,724    | 100        |
| RNA genes                       | 80       | 1.7        |
| Protein-coding genes            | 4,644    | 98.3       |
| Genes with function prediction  | 3,181    | 68.5       |
| Genes assigned to COGs          | 3,710    | 80         |
| Genes with peptide signals      | 372      | 8          |
| Genes with transmembrane helices| 702      | 15.1       |

aThe total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

Table 4. Number of genes associated with the 25 general COG functional categories

| Code | Value | %age | Description                  |
|------|-------|------|------------------------------|
| J    | 175   | 3.77 | Translation                  |
| A    | 1     | 0.02 | RNA processing and modification |
| K    | 387   | 8.33 | Transcription                |
| L    | 169   | 3.64 | Replication, recombination and repair |
| B    | 34    | 0.73 | Cell cycle control, mitosis and meiosis |
| Y    | 0     | 0    | Nuclear structure            |
| V    | 46    | 0.99 | Defense mechanisms           |
| T    | 176   | 3.79 | Signal transduction mechanisms |
| M    | 237   | 5.10 | Cell wall/membrane biogenesis |
| N    | 143   | 3.08 | Cell motility                |
| Z    | 0     | 0    | Cytoskeleton                 |

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Table 4 (cont.). Number of genes associated with the 25 general COG functional categories

| Code | Value | %age | Description |
|------|-------|------|-------------|
| W    | 0     | 0    | Extracellular structures |
| U    | 143   | 3.08 | Intracellular trafficking and secretion |
| O    | 138   | 2.97 | Posttranslational modification, protein turnover, chaperones |
| C    | 218   | 4.69 | Energy production and conversion |
| G    | 472   | 10.16| Carbohydrate transport and metabolism |
| E    | 463   | 9.97 | Amino acid transport and metabolism |
| F    | 79    | 1.70 | Nucleotide transport and metabolism |
| H    | 175   | 3.77 | Coenzyme transport and metabolism |
| I    | 112   | 2.41 | Lipid transport and metabolism |
| P    | 305   | 6.57 | Inorganic ion transport and metabolism |
| Q    | 99    | 2.13 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 563   | 12.12| General function prediction only |
| S    | 363   | 7.82 | Function unknown |
| -    | 934   | 20.11| Not in COGs |

The total is based on the total number of protein coding genes in the annotated genome.

Conclusion

On the basis of phenotypic, phylogenetic and genomic analyses, we formally propose the creation of Enterobacter massiliensis sp. nov. that contains strain JC163T. This bacterium was cultivated from a healthy Senegalese individual, from whom several other previously undescribed bacterial species were also cultivated through diversification of culture conditions [4-15], thus suggesting that the human fecal flora from humans remains partially unknown.

Description of Enterobacter massiliensis sp. nov.

Enterobacter massiliensis (mas.i.li.en'sis. L. gen. masc. n. massiliensis, of Massilia, the Latin name of Marseille where strain JC163T was first isolated and cultivated).

Colonies are 2 mm in diameter on Brain-Heart Infusion agar and are convex, opaque, light-cream colored and circular with regular margins. Cells are rods with tufts of polar flagella and a mean diameter of 1.02 µm and a mean length of 1.90 µm. Optimal growth is achieved in an aerobic atmosphere supplemented with 5% CO2. Weak growth is observed in microaerophilic conditions. No growth is observed under anaerobic conditions in the absence of CO2. Growth occurs between 25 and 45°C, with optimal growth occurring between 30 and 37°C. Cells stain Gram-negative, are non-endospore forming and are motile. Cells are positive for catalase and indole production. β-galactosidase and glucose, mannitol, sorbitol and rhamnose fermentation activities are present. Nitrate reduction, urease and oxidase activities are absent. Cells are susceptible to ticarcillin, imipenem, trimethoprim/sulfamethoxazole, gentamicin, amikacin, and colimycin, but resistant to fosfomycin and nitrofurantoin. The G+C content of the genome is 55.1%. The 16S rRNA and genome sequences are deposited in Genbank and EMBL under accession numbers JN657217 and CAEO000000000, respectively. The type strain JC163T (= CSUR P161 = DSM 26120) was isolated from the fecal flora of a healthy patient in Senegal.

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References

1. Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournier G, Gimenez G, Maraninchi M, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. [In press]. Clin Microbiol Infect 2012; 18:1185-1193. PubMed

2. Genome Online Database. http://www.genomesonline.org/cgi-bin/GOLD/index.cgi

3. Tindall BJ, Rossello-Mora R, Busse HJ, Ludwig W, Kampfer P. Notes on the characterization of prokaryote strains for taxonomic purposes. Int J Syst Evol Microbiol 2010; 60:249-266. PubMed http://dx.doi.org/10.1099/ijs.0.016949-0

4. Kokcha S, Mishra AK, Lagier JC, Million M, Leroy Q, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Bacillus timonensis sp. nov. Stand Genomic Sci 2012; 6:346-355. PubMed http://dx.doi.org/10.4056/sigs.2776064

5. Lagier JC, El Karkouri K, Nguyen TT, Armougom F, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Anaerococcus senegalensis sp. nov. Stand Genomic Sci 2012; 6:116-125. PubMed http://dx.doi.org/10.4056/sigs.2415480

6. Mishra AK, Gimenez G, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Alistipes senegalensis sp. nov. Stand Genomic Sci 2012; 6:304-314. http://dx.doi.org/10.4056/sigs.2625821

7. Lagier JC, Armougom F, Mishra AK, Nguyen TT, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Alistipes timonensis sp. nov. Stand Genomic Sci 2012; 6:315-324. PubMed http://dx.doi.org/10.4056/sigs.2685917

8. Mishra AK, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Clostridium senegalense sp. nov. Stand Genomic Sci 2012; 6:386-395. PubMed

9. Mishra AK, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Peptoniphilus timonensis sp. nov. Stand Genomic Sci 2012; 7:1-11. http://dx.doi.org/10.4056/sigs.2956294

10. Mishra AK, Lagier JC, Rivet R, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Paenibacillus senegalensis sp. nov. Stand Genomic Sci 2012; 7:70-81. http://dx.doi.org/10.4056/sigs.3054650

11. Lagier JC, Gimenez G, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Herbaspirillum massiliense sp. nov. Stand Genomic Sci 2012; 7:200-209. PubMed http://dx.doi.org/10.4056/sigs.3086474

12. Roux V, El Karkouri K, Lagier JC, Robert C, Raoult D. Non-contiguous finished genome sequence and description of Kurthia massiliensis sp. nov. Stand Genomic Sci 2012; 7:221-232. PubMed http://dx.doi.org/10.4056/sigs.3206554

13. Kokcha S, Ramasamy D, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Brevibacterium senegalense sp. nov. Stand Genomic Sci 2012; 7:233-245. PubMed http://dx.doi.org/10.4056/sigs.3256677

14. Ramasamy D, Kokcha S, Lagier JC, N’Guyen TT, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Aeromicrobium massilense sp. nov. Stand Genomic Sci 2012; 7:246-257. PubMed http://dx.doi.org/10.4056/sigs.3306717

15. Lagier JC, Ramasamy D, Rivet R, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Cellulomonas massiliensis sp. nov. Stand Genomic Sci 2012; 7:258-270. PubMed http://dx.doi.org/10.4056/sigs.3316719

16. Hormaeche E, Edwards PR. A proposed genus Enterobacter. Int Bull bacteriol Nomen Taxon 1960; 10:71-74

17. List of Prokaryotic names with Standing in Nomenclature. http://www.bacterio.cict.fr

18. Izard D, Gavini F, Trinel PA, Leclerc H. Deoxyribonucleic acid relatedness between Enterobacter cloacae and Enterobacter amnigenus sp. nov. Int J Syst Bacteriol 1981; 31:35-42. http://dx.doi.org/10.1099/00207713-31-1-35

19. Madhaiyan M, Poonguzhali S, Lee JS, Saravanavan VS, Lee KC, Santhanakrishnan P. Enterobacter arachidis sp. nov., a plant-growth-promoting diazotrophic bacterium isolated from rhizosphere soil of groundnut. Int J Syst Evol Microbiol 2010; 60:1559-1564. PubMed http://dx.doi.org/10.1099/ijs.0.013664-0
Enterobacter massiliensis sp. nov.

20. Brenner DJ, McWhorter AC, Kai A, Steigerwalt AG, Farmer JJ, III. *Enterobacter asburiae* sp. nov., a new species found in clinical specimens, and reassignment of *Erwinia dissolvens* and *Erwinia nimipressuralis* to the genus *Enterobacter* as *Enterobacter dissolvens* comb. nov. and *Enterobacter nimipressuralis* comb. nov. *J Clin Microbiol* 1986; 23:1114-1120. PubMed

21. Dickey RS, Zumoff CH. Emended description of *Enterobacteriaceae* sp. nov. (formerly *Erwinia cancerogenus*). *Int J Syst Bacteriol* 1988; 38:371-374. http://dx.doi.org/10.1099/00207713-38-4-371

22. Hoffmann H, Stindl S, Ludwig W, Stumpf A, Mehlen A, Heesemann J, Monet D, Schleifer KH, Roggenkamp A. Reassignment of *Enterobacter dissolvens* to *Enterobacter cloacae* as *E. cloacae* subspecies *dissolvens* comb. nov. and emended description of *Enterobacter asburiae* and *Enterobacter kobei*. *Syst Appl Microbiol* 2005; 28:196-205. PubMed http://dx.doi.org/10.1016/j.syapm.2004.12.010

23. Inoue K, Sugiyama K, Kosako Y, Sakazaki R, Yamai S. *Enterobacter cowanii* sp. nov., a new species of the family *Enterobacteriaceae*. *Curr Microbiol* 2000; 41:417-420. PubMed http://dx.doi.org/10.1007/s002840010160

24. Brenner DJ, Richard C, Steigerwalt AG, Asbury MA, Mandel M. *Enterobacter gergoviae* sp. nov.: a new species of *Enterobacteriaceae* found in clinical specimens and the environment. *Int J Syst Bacteriol* 1980; 30:1-6. http://dx.doi.org/10.1099/00207713-30-1-1

25. Stephan R, Van TS, Cleenwerck I, Iversen C, Stephan R, Van TS, Cleenwerck I, Iversen C. *Enterobacter turicensis* sp. nov., isolated from fruit powder, infant formula and an infant formula production environment. *Int J Syst Microbiol* 2011; 61:2769-2774. PubMed http://dx.doi.org/10.1099/00207713-0

26. O'Hara CM, Farmer JJ, III. *Enterobacter cloacae* sp. nov.: a lignin-degrading gamma-proteobacteria isolated from soil. *Curr Microbiol* 1980; 56:569-584. http://dx.doi.org/10.1007/0-07-0207713-0-3-569

27. Kosako Y, Tamura K, Sakazaki R, Miki K. *Enterobacter kobei* sp. nov., a new species of the family *Enterobacteriaceae* resembling *Enterobacter cloacae*. *Curr Microbiol* 1996; 33:261-265. PubMed http://dx.doi.org/10.1007/s002849900110

28. Hoffmann H, Stindl S, Stumpf A, Mehlen A, Monet D, Heesemann J, Schleifer KH, Roggenkamp A. Description of *Enterobacter ludwigii* sp. nov., a novel *Enterobacter* species of clinical relevance. *Syst Appl Microbiol* 2005; 28:206-212. PubMed http://dx.doi.org/10.1016/j.syapm.2004.12.009

29. Zhu B, Lou MM, Xie GL, Wang GF, Zhou Q, Wang F, Fang Y, Su T, Li B, Duan YP. *Enterobacter mori* sp. nov., associated with bacterial wilt on *Morus alba* L. *Int J Syst Evol Microbiol* 2011; 61:2769-2774. PubMed http://dx.doi.org/10.1099/00207713-0

30. Peng G, Zhang W, Luo H, Xie H, Lai W, Tan Z. *Enterobacter oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the wild rice species *Oryza latifolia*. *Int J Syst Evol Microbiol* 2009; 59:1650-1655. PubMed

31. Stephan R, Van TS, Cleenwerck I, Iversen C, Joosten H, De VP, Lehner A. *Enterobacter pulveris* sp. nov., isolated from fruit powder, infant formula and an infant formula production environment. *Int J Syst Microbiol* 2008; 58:237-241. PubMed http://dx.doi.org/10.1099/00207713-0

32. Chung YR, Brenner DJ, Steigerwalt AG, Kim BS, Kim HT, Cho KY. *Enterobacter pyrinus* sp. nov., an organism associated with brown leaf spot disease of pear trees. *Int J Syst Bacteriol* 1993; 43:157-161. http://dx.doi.org/10.1099/00207713-43-1-157

33. Kämpfer P, Ruppel S, Remus R. *Enterobacter radicincitans* sp. nov., a plant growth promoting species of the family *Enterobacteriaceae*. *Syst Appl Microbiol* 2005; 28:213-221. PubMed http://dx.doi.org/10.1016/j.syapm.2004.12.007

34. Farmer JJ, Asbury MA, Hickman FW, Brenner DJ. *Enterobacter sakazakii*: a new species of *Enterobacteriaceae* isolated from clinical specimens. *Int J Syst Bacteriol* 1980; 30:569-584. http://dx.doi.org/10.1099/00207713-30-3-569

35. Manter DK, Hunter WJ, Vivanco JM. *Enterobacter soli* sp. nov.: a lignin-degrading gamma-proteobacteria isolated from soil. *Curr Microbiol* 2011; 62:1044-1049. PubMed http://dx.doi.org/10.1007/s00284010-9809-9

36. Mezzatesta ML, Gona F, Stefani S. *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. [PubMed]. *Future Microbiol* 2012; 7:887-902. PubMed http://dx.doi.org/10.2217/fmb.12.61

37. Trape JF, Tall A, Diagne N, Ndiath O, Ly AB, Faye J, Dieye-Ba F, Roucher C, Bouganali C,
Badiane A, et al. Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study. *Lancet Infect Dis* 2011; 11:925-932. PubMed http://dx.doi.org/10.1016/S1473-3099(11)70194-3

38. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eukarya. *Proc Natl Acad Sci USA* 1990; 87:4576-4579. PubMed http://dx.doi.org/10.1073/pnas.87.12.4576

39. Garrity GM, Bell JA, Lilburn T. Phylum XIV. *Proteobacteria* phyl. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT (eds), Bergey’s Manual of Systematic Bacteriology, Second Edition, Volume 2, Part B, Springer, New York, 2005, p. 1.

40. Validation list No. 106. *Int J Syst Evol Microbiol* 2005; 55:2235-2238. http://dx.doi.org/10.1099/ijsem.0.64108-0

41. Garrity GM, Bell JA, Lilburn T. Class III. *Gammaproteobacteria* class. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds), Bergey’s Manual of Systematic Bacteriology, Second Edition, Volume 2, Springer, New York, 2005 p. 1.

42. Garrity GM, Holt JG. Taxonomic Outline of the *Archaea* and *Bacteria*. In: Garrity GM, Boone DR, Castenholz RW (eds), Bergey’s Manual of Systematic Bacteriology, Second Edition, Volume 1, Springer, New York, 2001, p. 155-166.

43. Rahn O. New principles for the classification of bacteria. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung II* 1937; 96:273-286.

44. Skerman VBD, Sneath PHA. Approved list of bacterial names. *Int J Syst Bacteriol* 1980; 30:225-420. http://dx.doi.org/10.1099/00207713-30-1-225

45. Judicial Commission. Conservation of the family name *Enterobacteriaceae*, of the name of the type genus, and designation of the type species OPIN-ION NO. 15. *Int Bull Bacteriol Nomencl Taxon* 1958; 8:73-74.

46. Sakazaki R. Genus VII. *Enterobacterium* Hormaeche and Edwards 1960, 72; Nom. cons. Opin. 28, Jud. Comm. 1963, 38. In: Buchanan RE, Gibbons NE (eds), Bergey’s Manual of Determinative Bacteriology, Eighth Edition, The Williams and Wilkins Co., Baltimore, 1974, p. 324-325.

47. Judicial Commission. Conservation of the family name *Enterobacteriaceae*, of the name of the type genus, and designation of the type species OPIN-ION NO. 15. *Int Bull Bacteriol Nomencl Taxon* 1958; 8:73-74.

48. Editorial Board. OPINION 28 Rejection of the bacterial generic name *Cloaca* Castellani and Chalmers and acceptance of *Enterobacter* Hormaeche and Edwards as a bacterial generic name with type species *Enterobacter cloacae* (Jordan) Hormaeche and Edwards. *Int Bull Bacteriol Nomencl Taxon* 1963; 13:38.

49. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; 25:25-29. PubMed http://dx.doi.org/10.1038/75556

50. Sillankorva S, Oliveira D, Moura A, Henriques M, Faustino A, Nicolau A, Azeredo J. Efficacy of a broad host range lytic bacteriophage against E. coli adhered to urothelium. *Curr Microbiol* 2011; 62:1128-1132. PubMed http://dx.doi.org/10.1007/s00284-010-9834-8

51. Stackebrandt E, Ebers J. Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 2006; 33:152-155.

52. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. Ongoing revaluation in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009; 49:543-551. PubMed http://dx.doi.org/10.1086/600885

53. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV, et al. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* 2008; 26:541-547. PubMed http://dx.doi.org/10.1038/nbt1360

54. Prodigal. http://prodigalornl.gov

55. GenBank database. http://www.ncbi.nlm.nih.gov/genbank

56. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 1997; 25:955-964. PubMed

57. Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. Rfammer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007; 35:3100-3108. PubMed http://dx.doi.org/10.1093/nar/gkm160

http://standardsingenomics.org
Enterobacter massiliensis sp. nov.

58. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 2004; **340**:783-795. PubMed [http://dx.doi.org/10.1016/j.jmb.2004.05.028](http://dx.doi.org/10.1016/j.jmb.2004.05.028)

59. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol* 2001; **305**:567-580. PubMed [http://dx.doi.org/10.1006/jmbi.2000.4315](http://dx.doi.org/10.1006/jmbi.2000.4315)