Non-contiguous finished genome sequence and description of Corynebacterium jeddahense sp. nov.

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Corynebacterium jeddahense sp. nov., strain JCB¹, is the type strain of Corynebacterium jeddahense sp. nov., a new species within the genus Corynebacterium. This strain, whose genome is described here, was isolated from fecal flora of a 24-year-old Saudi male suffering from morbid obesity. Corynebacterium jeddahense is a Gram-positive, facultative anaerobic, nonsporulating bacillus. Here, we describe the features of this bacterium, together with the complete genome sequencing and annotation, and compare it to other member of the genus Corynebacterium. The 2,472,125 bp-long genome (1 chromosome but not plasmid) contains 2,359 protein-coding and 53 RNA genes, including 1 rRNA operon.

Introduction

Corynebacterium jeddahense strain JCB¹ (= CSUR P778 = DSM 45997) is the type strain of C. jeddahense sp. nov. This bacterium is a Gram-positive bacillus, non-spore-forming, strictly aerobic and non-motile that was isolated from the feces of a 24 year-old man living in Jeddah, Saudi Arabia, who suffered from morbid obesity. This isolation was part of a “culturomics” study aiming at cultivating the maximum number of bacterial species from human feces [1,2].

The current classification of bacteria remains a matter of debate and relies on a combination of phenotypic and genomic characteristics [3]. Currently, more than 12,000 bacterial genomes have been sequenced [4], and we recently proposed an innovative concept for the taxonomic description of new bacterial species that integrates their genomic characteristics [5-35] as well as proteomic information obtained by MALDI-TOF-MS analysis [36].

In the present study, we present a summary classification and a set of features for Corynebacterium jeddahense sp. nov., strain JCB¹ (CSUR P778 = DSM 45997), including the description of its complete genome sequence and annotation. These characteristics support the circumscription of the species Corynebacterium jeddahense. The genus Corynebacterium was created in 1896 by Lehmann and Neumann and currently consists of mainly Gram-positive, non-spore-forming, rod-shaped bacteria with a high DNA G+C content [37]. This genus belongs to the phylum Actinobacteria and currently includes more than 100 species with standing in nomenclature [38]. Members of the genus Corynebacterium are found in various environments including water, soil, sewage, and plants.
as well as in human normal skin flora and human or animals clinical samples. Some *Corynebacterium* species are well-established human pathogens while others are only considered as opportunistic pathogens. *Corynebacterium diptheriae*, causing diphtheria, is the most significant pathogen in this genus [39]. However, many *Corynebacterium* species including, among others, *C. jeikeium*, *C. urealyticum*, *C. striatum*, *C. ulcerans* and *C. pseudotuberculosis*, are recognized agents of bacteremias, endocarditis, urinary tract infections, and respiratory or wound infections [40].

### Classification and features

A stool sample was collected from a 24-year-old man living in Jeddah, Saudi Arabia, who suffered from morbid obesity (BMI=52). The patient gave a signed informed consent. The study and the assent procedure were approved by the Ethics Committees of the King Abdulaziz University, King Fahd medical Research Center, Saudi Arabia, under agreement number 014-CEGMR-2-ETH-P, and of the Institut Fédératif de Recherche 48, Faculty of Medicine, Marseille, France, under agreement number 09-022. The patient was not taking any antibiotics at the time of stool sample collection and the fecal sample was kept at -80°C after collection. Strain JCB (Table 1) was first isolated in July 2013 by cultivation on 5% sheep blood-enriched Columbia agar (BioMerieux, Marcy l’Etoile, France) in aerobic atmosphere with 5% CO₂ at 37°C after a 14-day preincubation of the stool sample in an aerobic blood culture bottle that also contained sterile rumen sheep fluid. Several other new bacterial species were isolated from this stool specimen using various culture conditions.

**Table 1.** Classification and general features of *C. jeddahense* strain JCB according to the MIGS recommendations [41].

| MIGS ID | Property                  | Term                      | Evidence code³ |
|---------|---------------------------|---------------------------|----------------|
| MIGS-6.3| Salinity                  | Unknown                   | IDA            |
| MIGS-22 | Oxygen requirement        | Aerobic                   | IDA            |
| MIGS-15 | Biotic relationship       | Free living               | IDA            |
| MIGS-14 | Pathogenicity             | Unknown                   | IDA            |
| MIGS-4  | Geographic location       | Jeddah, Saudi Arabia      | IDA            |
| MIGS-5  | Sample collection time    | July 2013                 | IDA            |
|         | Latitude                  | 21.422487                 |                |
| MIGS-4.1| Longitude                 | 39.826184                 | IDA            |
| MIGS-4.3| Depth                     | Surface                   | IDA            |
| MIGS-4.4| Altitude                  | 0 m above sea level       | IDA            |

³Evidence 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 [51]. 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.
This strain exhibited a 96.8% nucleotide sequence similarity with *C. coyleae*, the phylogenetically most closely related *Corynebacterium* species with a validly published name (Figure 1). The similarity 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 [52], and was in the 82.9 to 99.60% range observed among members of the genus *Corynebacterium* with standing in the nomenclature [53].

Four growth temperatures (25, 30, 37, 45°C) were tested. Growth occurred between 30 and 45°C on blood-enriched Columbia agar (BioMerieux), with the optimal growth being obtained at 37°C after 48 hours of incubation. 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 achieved aerobically. Weak cell growth was observed under microaerophilic and anaerobic conditions. The motility test was negative and the cells were not sporulating. Colonies were translucent and 1 mm in diameter on blood-enriched Columbia agar. Cells were Gram-positive rods (Figure 2). In electron microscopy, the bacteria grown on agar had a mean diameter and length of 0.63 and 1.22 μm, respectively (Figure 3).

Figure 1. Phylogenetic tree highlighting the position of *Corynebacterium jeddahense* strain JCBᵀ relative to other type strains within the genus *Corynebacterium*. GenBank accession numbers are indicated in parentheses. Sequences were aligned using CLUSTALW, and phylogenetic inferences obtained using the maximum-likelihood method in the MEGA software package. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. *Mycobacterium avium* was used as outgroup. The scale bar represents 1% nucleotide sequence divergence.
Strain JCB<sup>T</sup> was catalase positive and oxidase negative. Using an API CORYNE strip, a positive reaction was observed only for alkaline phosphatase and for catalase. Negative reactions were observed for reduction of nitrates, pyridoxal arylamidase, pyrazinamidase, β-glucuronidase, β-galactosidase, α-glucosidase N-acetylβ-glucosaminidase, β-glucosidase, urease, gelatin hydrolysis and fermentation of glucose, ribose xylose, mannitol, maltose, lactose, saccharose and glycojen. Using the Api Zym system (BioMerieux), alkaline and acid phosphatases and Naphtol-AS-BI phosphohydrolase activities were positive, but esterase (C4), esterase lipase (C8), lipase (C14), trypsin, α-chemotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, N-acetylβ-glucosaminidase, leucine arylamidase, valine arylamidase, cystin arylamidase, α-mannosidase and α-fucosidase activities were negative.

Substrate oxidation and assimilation were examined with an API 50CH strip (BioMerieux) at 37°C. All reactions were negative, including fermentation of starch, glycogen, glycerol, erythritol, esculin ferric citrate, amygdalin, arbutin, salicin, L-arabinose, D-ribose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, methyl α-D-xylopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-raffinose, D-lyxose, D-arabinose, L-lyxose, D-adenitol, L-sorbose, dulcitol, inositol, D-sorbitol, D-melezitose, D-xylitol, gentiobiose, D-turanose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, and potassium 2-ketogluconate.

*C. jeddahense* is susceptible to amoxicillin, ceftriaxone, imipenem, rifampin, gentamicin, doxycycline and vancomycin, but resistant to ciprofloxacin, trimethoprim/sulfamethoxazole, erythromycin and metronidazole. When compared with representative species from the genus *Corynebacterium*, *C. jeddahense* strain JCB<sup>T</sup> exhibited the phenotypic differences detailed in Table 2.
### Table 2. Differential characteristics of *C. jeddahense* strain JCB\(^7\) and closely related strains.

|                | *C. jeddahense* | *C. pseudotuberculosis* | *C. efficiens* | *C. glutamicum* | *C. lipophiloflavum* | *C. coyleae* | *C. glaucum* |
|----------------|-----------------|-------------------------|----------------|------------------|----------------------|-------------|-------------|
| **Size (μm)**  | 0.63 x 1.22     | 0.5-0.6 x 1.0-3.3       | 0.8-1.1 x 1.0-4.5 | 0.7-1 x 1-3     | NA                   | NA          | NA          |
| **O\(_2\) requirement** | Aero-anaerobic | Aero-anaerobic | Aero-anaerobic | Aero-anaerobic | Pale yellow to yellow | Yellow | None | Light anaerobic |
| **Pigment production** | None | Yellowish-white | Yellow | Yellow | Yellow | Yellow | None | Light grey |
| **Gram stain**  | +               | +                       | +             | +                | +                    | +           | +           |
| **Motility**    | -               | -                       | -             | -                | -                    | -           | -           |
| **Endospore formation** | - | - | - | - | - | - | - |
| **Production of** |               |                          |               |                  |                      |             |             |
| Acid phosphatase | +               | NA                      | NA            | -                | +                    | +           | +           |
| Alkaline phosphatase | +              | V                       | NA            | -                | +                    | +           | +           |
| Catalase        | +               | +                       | +             | +                | +                    | +           | +           |
| Oxidase         | -               | -                       | -             | -                | -                    | -           | -           |
| Pyrazinamidase  | -               | -                       | +             | -                | +                    | -           | +           |
| Nitrate reductase | -               | V                       | +             | +                | -                    | -           | -           |
| Urease          | -               | +                       | V             | +                | W                    | -           | -           |
| **Utilization of** |               |                          |               |                  |                      |             |             |
| Ribose          | -               | +                       | +             | -                | +                    | -           | -           |
| Mannose         | -               | +                       | +             | +                | NA                   | -           | -           |
| Mannitol        | -               | -                       | -             | -                | +                    | -           | -           |
| Sucrose         | -               | +                       | +             | +                | -                    | +           | +           |
| D-glucose       | -               | +                       | +             | +                | -                    | +           | +           |
| D-fructose      | -               | +                       | +             | +                | NA                   | -           | NA          |
| D-maltose       | -               | +                       | +             | +                | -                    | +           | -           |
| D-lactose       | -               | -                       | -             | -                | -                    | -           | -           |
| **Habitat**     | Human gut       | Sheep, infected gland,  | Soil, Japan   | Sewage, Japan    | vaginal swab,        | Human blood | Cosmetic dye |
| **Optimal temp (°C)** | 37°C             | 37°C                    | 30-40°C       | 25-37°C          | 37°C                 | 37°C        | 37°C        |

+ : Positive; - : negative; V : variable; W : weak reaction; NA : not available.

*C. pseudotuberculosis* strain CIP 102968\(^T\) [54], *C. efficiens* YS-314\(^T\) [55], *C. glutamicum* strain ATCC 13032\(^T\) [56], *C. lipophiloflavum* strain DSM 44291\(^T\) [57], *C. coyleae* strain DSM44184\(^T\) [58] and *C. glaucum* strain IMMIB R-5091\(^T\) [59].
(MALDI-TOF) MS protein analysis was carried out as previously described [36] using a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany). Twelve individual colonies were deposited on a MTP 384 MALDI-TOF target plate (Bruker). The twelve 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 4,706 bacteria, including 169 spectra from 69 validly named Corynebacterium species used as reference data in the BioTyper database. The score generated enabled the presumptive identification and discrimination of the tested species from those in a database: a score > 2 with a validated species enabled the identification at the species level; and a score < 1.7 did not enable any identification. For strain JCB\textsuperscript{T}, no significant score was obtained, suggesting that our isolate was not a member of any known species (Figures 4 and 5).

![Figure 4](image1.png)

**Figure 4.** Reference mass spectrum from *C. jeddahense* strain JCB\textsuperscript{T}. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

![Figure 5](image2.png)

**Figure 5.** Gel view comparing *C. jeddahense* strain JCB\textsuperscript{T} (= CSUR P778 = DSM 45997) to other species from the genus *Corynebacterium*. The gel view displays the raw spectra of loaded spectrum files as a pseudo-electrophoretic gel. 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 grey scale scheme code. The grey scale bar on the right y-axis indicates the relation between the shade of grey of the “band” and the peak intensity, in arbitrary units. Displayed species are indicated on the left.
Figure 5 (cont.). Gel view comparing *C. jeddahense* strain JCB\(^T\) (= CSUR P778 = DSM 45997) to other species from the genus *Corynebacterium*. The gel view displays the raw spectra of loaded spectrum files as a pseudo-electrophoretic gel. 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 grey scale scheme code. The grey scale bar on the right y-axis indicates the relation between the shade of grey of the “band” and the peak intensity, in arbitrary units. Displayed species are indicated on the left.

**Genome sequencing information**

**Genome project history**

The organism was selected for sequencing on the basis of its phylogenetic position, 16S rDNA similarity and phenotypic differences with members of the genus *Corynebacterium* and is part of a culturomics study of the human digestive flora aiming at isolating all bacterial species within human feces [2]. It was the 96th genome from a *Corynebacterium* species. The EMBL accession number is CBYN00000000 and consists of 244 contigs. Table 3 shows the project information and its association with MIGS version 2.0 compliance [41].

**Table 3. Project information**

| MIGS ID   | Property                          | Term                                      |
|-----------|-----------------------------------|-------------------------------------------|
| MIGS-31   | Finishing quality                 | High-quality draft                        |
| MIGS-28   | Libraries used                    | One paired-end 454 3-kb library           |
| MIGS-29   | Sequencing platforms              | 454 GS FLX Titanium                       |
| MIGS-31.2 | Fold coverage                     | 130                                       |
| MIGS-30   | Assemblers                        | Newbler version 2.5.3                     |
| MIGS-32   | Gene calling method               | Prodigal                                  |
|           | BioProject ID                     | PRJEB4941                                 |
| GenBank Accession number |                          | CBYN00000000                              |
| GenBank date of release |                          | February 12, 2014                          |
|           | Project relevance                 | Study of the human gut                    |

Growth conditions and DNA isolation

*C. jeddahense* sp. nov strain JCB\(^T\) (= CSUR P778 = DSM 45997) was grown aerobically on sheep blood-enriched Columbia agar medium at 37°C. Two petri dishes were spread and resuspended in 6x100μl of G2 buffer (EZI DNA Tissue Kit, Qiagen). A first mechanical lysis was performed using glass powder on the Fastprep-24 device (Sample Preparation System, MP Biomedicals, USA) using 2x20 second bursts. DNA was treated with 2.5μg/μL of
lysozyme for 30 minutes at 37°C) and extracted using the BioRobot EZ 1 Advanced XL (Qiagen). The DNA was then concentrated and purified on a Qiaamp kit (Qiagen). The DNA concentration, as measured by the Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA), was 3.1ng/µl.

Genome sequencing and assembly
Genomic DNA of C. jeddahense was sequenced on a MiSeq sequencer (Illumina Inc, San Diego, CA, USA) using both paired-end and mate-pair sequencing with the Nextera XT DNA sample and Nextera Mate Pair sample prep kits, respectively (Illumina).

To prepare the paired-end library, Genomic DNA was diluted 1:3 to obtain a 1ng/µl concentration. The “tagmentation” step fragmented and tagged the DNA with a mean size of 1.4kb. Then, a limited PCR amplification (12 cycles) completed the tag adapters and introduced dual-index barcodes. After purification on AMPure XP beads (Beckman Coulter Inc, Fullerton, CA, USA), the library was then normalized on specific beads according to the Nextera XT protocol (Illumina). The pooled single strand library was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and paired end sequencing with dual index reads were performed in a single 39-hours run in 2x250-bp. Total information of 5.3Gb was obtained from a 574 K/mm² cluster density with a cluster passing quality control filters of 95.4% (11,188,000 clusters). Within this run, the index representation for Corynebacterium jeddahense was determined to 6.2%. The 641,099 reads were filtered according to the read qualities. Genome assembly was performed using Newbler (Roche).

Genome annotation
Open Reading Frames (ORFs) were predicted using Prodigal [60] with default parameters. However, the predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against GenBank [61] and Clusters of Orthologous Groups (COG) databases using BLASTP. The tRNAs and rRNAs were predicted using the tRNAscanSE [62] and RNAmmer [63] tools, respectively. Lipoprotein signal peptides and numbers of transmembrane helices were predicted using SignalP [64] and TMHMM [65], respectively. ORFans were identified if their BLASTP E-value was lower than 1e⁻³ for alignment length greater than 80 amino acids. If alignment lengths were smaller than 80 amino acids, we use an E-value of 1e⁻⁵. Such parameter thresholds have already been used in previous works to define ORFans. Artemis [66] and DNA Plotter [67] were used for data management and visualization of genomic features, respectively. The Mauve alignment tool (version 2.3.1) was used for multiple genomic sequence alignments [68]. To estimate the mean level of nucleotide sequence similarity at the genome level between C. jeddahense and another 4 members of the Corynebacterium genus (Tables 6 and 7), we used the Average Genomic Identity Of gene Sequences (AGIOS) home-made software [35]. Briefly, this software combines the Proteinortho software [69] for detecting orthologous proteins between genomes compared two by two, then retrieves the corresponding genes and determines the mean percentage of nucleotide sequence identity among orthologous ORFs using the Needleman-Wunsch global alignment algorithm.
Genome properties

The genome \textit{C. jeddahense} strain JCB\(^r\) is 2,472,125 bp long (one chromosome, no plasmid) with a G+C content of 67.2\% (Figure 6, Table 4). Of the 2,412 predicted chromosomal genes, 2,359 were protein-coding genes and 53 were RNAs. A total of 1,462 genes (60.61\%) were assigned a putative function. Sixty-seven genes were identified as ORF\(\text{s}\) (2.77\%) and the remaining genes were annotated as hypothetical proteins. The properties and statistics of the genome are summarized in Table 4. The distribution of genes into COGs functional categories is presented in Table 5.

| Table 4. Nucleotide content and gene count levels of the Chromosome |
|------------------|------------------|----------|
| **Attribute**    | **Value**        | **% of total** |
| Genome size (bp) | 2,472,125        |           |
| DNA G+C content (bp) | 1,661,268      | 67.2     |
| DNA coding region (bp) | 2,235,018  | 87.17    |
| Extrachromosomal elements | 0            |          |
| Total genes      | 2,412            | 100      |
| RNA genes        | 53               | 2.2      |
| Protein-coding genes | 2,359        | 97.8     |
| Genes with function prediction | 1,462 | 60.61 |
| Genes assigned to COGs | 1,636       | 67.82 |
| Genes with peptide signals | 187         | 7.75    |
| Genes with transmembrane helices | 629        | 26.1    |

\(^{a}\) The 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 5. Number of genes associated with the 25 general COG functional categories |
|------------------|------------------|----------|
| **Code** | **Value** | **% age\(^a\)** | **Description** |
| J | 149 | 6.18 | Translation |
| A | 1 | 0.04 | RNA processing and modification |
| K | 132 | 5.47 | Transcription |
| L | 154 | 6.38 | Replication, recombination and repair |
| B | 0 | 0 | Chromatin structure and dynamics |
| D | 22 | 0.91 | Cell cycle control, mitosis and meiosis |
| Y | 0 | 0 | Nuclear structure |
| V | 32 | 1.32 | Defense mechanisms |
| T | 57 | 2.36 | Signal transduction mechanisms |
| M | 104 | 4.31 | Cell wall/membrane biogenesis |
| N | 1 | 0.04 | Cell motility |
| Z | 0 | 0 | Cytoskeleton |
| W | 0 | 0 | Extracellular structures |
| U | 21 | 0.87 | Intracellular trafficking and secretion |
| | | | Posttranslational modification, protein turnover, chaperones |
| O | 58 | 2.2 | Energy production and conversion |
| C | 85 | 3.52 | Carbohydrate transport and metabolism |
| G | 109 | 4.52 | Amino acid transport and metabolism |
| E | 191 | 7.1 | Nucleotide transport and metabolism |
| H | 85 | 3.52 | Coenzyme transport and metabolism |
| I | 47 | 1.95 | Lipid transport and metabolism |
| P | 135 | 5.6 | Inorganic ion transport and metabolism |
| Q | 40 | 1.66 | Secondary metabolites biosynthesis, transport and catabolism |
| R | 232 | 9.62 | General function prediction only |
| S | 145 | 6.01 | Function unknown |
| - | 776 | 32.17 | Not in COGs |

\(^{a}\) The total is based on the total number of protein coding genes in the annotated genome
Corynebacterium jeddahense

Figure 6. Graphical circular map of the C. jeddahense strain JCB\textsuperscript{T} genome. From the outside in, the outer two circles show open reading frames oriented in the forward (colored by COG categories) and reverse (colored by COG categories) directions, respectively. The third circle marks the rRNA gene operon (red) and tRNA genes (green). The fourth circle shows the G+C% content plot. The inner-most circle shows GC skew, purple indicating negative values whereas olive for positive values.

Genome comparison of C. jeddahense with other Corynebacterium genomes

We compared the genome of C. jeddahense strain JCB\textsuperscript{T} with those of C. efficiens YS-314\textsuperscript{T}, C. lipophiloflavum strain DSM 44291\textsuperscript{T}, C. glutamicum strain ATCC 13032\textsuperscript{T} and C. pseudotuberculosis strain CIP 102968\textsuperscript{T} (Table 6 and 7). The draft genome sequence of C. jeddahense strain JCB\textsuperscript{T} is larger than those of C. efficiens, C. lipophiloflavum and C. glutamicum (2.47, 2.26, 2.43 and 2.11 Mb, respectively), but smaller than that of C. pseudotuberculosis (2.48 Mb). The G+C content of C. jeddahense is larger than those of C. efficiens, C. lipophiloflavum and C. glutamicum (67.2, 62.9, 64.8, 53.8, and 52.1%, respectively). The gene content of C. jeddahense (2,359) is smaller than those of C. efficiens, C. lipophiloflavum and C. glutamicum (2,398, 2,371 and 2,993, respectively) but larger than that of C. pseudotuberculosis (2,060). The distribution of genes into COG categories was similar but not identical in all four compared genomes (Figure 7).

In addition, C. jeddahense shared 1,369, 1,345, 1,385 and 1,230 orthologous genes with C. efficiens, C. lipophiloflavum, C. glutamicum and C. pseudotuberculosis, respectively. The AGIOS value ranged from 66.7 to 75.04 among compared Corynebacterium species except C. jeddahense. When compared to other species, the AGIOS value ranged from 66.44% with C. pseudotuberculosis to 77.26% with C. lipophiloflavum, thus confirming its new species status (Table 7).
Table 6. Genomic comparison of *C. jeddahense* and 4 other *Corynebacterium* species. †

| Species                    | Strain | Genome accession number | Genome size (Mb) | G+C content |
|----------------------------|--------|-------------------------|------------------|-------------|
| *C. jeddahense*            | JCB†   | CBYN000000000           | 2,472,125        | 67.2        |
| *C. efficiens*             | YS-314†| NC_004369               | 3,147,090        | 62.9        |
| *C. lipophiloflavum*       | DSM    |                         |                  |             |
| *C. glutamicum*            | 44291† | ACHJ000000000           | 2,293,743        | 64.8        |
| *C. pseudotuberculosis*    | CIP 52.97 | NC_017307          | 2,320,595        | 52.1        |

†Species name, strain, GenBank accession number, genome size and G+C content of compared genomes.

Table 7. Genomic comparison of *C. jeddahense* and 4 other *Corynebacterium* species. †

| C. jeddahense | C. efficiens | C. lipophiloflavum | C. glutamicum | C. pseudotuberculosis |
|---------------|--------------|--------------------|---------------|-----------------------|
| 2359          | 1,369        | 1,345              | 1,385         | 1,230                 |
| 71.81         | 2,938        | 1,449              | 1,605         | 1,381                 |
| 77.26         | 71.34        | 2,2371             | 1,465         | 1,285                 |
| 68.12         | 75.04        | 68.43              | 2,993         | 1,400                 |
| 66.44         | 67.93        | 66.7               | 68.47         | 2,060                 |

†Numbers of orthologous proteins shared between genomes (upper right); AGIOS values (lower left); numbers of proteins per genome (bold).

Figure 7. Distribution of functional classes of predicted genes in the genomes from *C. jeddahense* JCB† (colored in sea blue), *C. efficiens* YS-314† (blue), *C. lipophiloflavum* strain DSM 44291† (green), *C. glutamicum* strain ATCC 13032† (yellow) and *C. pseudotuberculosis* strain CIP 102968† (red) chromosomes, according to the clusters of orthologous groups of proteins.
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Conclusion
On the basis of phenotypic, phylogenetic and genomic analyses, we formally propose the creation of *Corynebacterium jeddahense* sp. nov., that contains the strain JCBT. The strain has been isolated from the fecal flora of a Saudi man suffering from morbid obesity. Several other as yet undescribed bacterial species were also cultivated from different fecal samples through diversification of culture conditions [5-35], thus suggesting that the human fecal flora of humans remains partially unknown.

Description of *Corynebacterium jeddahense* sp. nov.
*Corynebacterium jeddahense* (jed.dah.en’se N.L. neut. adj. Jeddah is the name of the town in Saudi Arabia where the specimen was obtained).

Grows occurred between 30 and 45°C on blood-enriched Columbia agar (BioMerieux). Optimal growth obtained at 37°C in aerobic atmosphere. Weak growth obtained in microaerophilic and anaerobic conditions. Colonies are translucent and 1 mm in diameter. Not motile, not endospore-forming. Cells are Gram-positive rods and have a mean diameter and length of 0.63 and 1.22 μm, respectively. Catalase positive, oxidase negative. Using API Coryne (BioMerieux), cells are alkaline phosphatase positive but negative for reduction of nitrates, pyrrolidonyl arylamidase, pyrazinamidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, leucine arylamidase, valine arylamidase, cystin arylamidase, α-mannosidase and α-fucosidase activities are negative. Using API 50CH (BioMerieux), fermentation of starch, glycerol, glycerol, erythritol, esculin ferric citrate, amygdalin, arbutin, salicin, L-arabinose, D-ribose, D-xyllose, methyl β-D-xyl-o-pyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, methyl α-D-xylopyranoside, methyl α-D-glucopyranoside, N-acetylgalcosamine, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-raffinose, D-lyxose, D-arabinose, L-xyllose, D-adonitol, L-sorbose, dulcitol, inositol, D-sorbitol, D-melezitose, D-xylitol, gentiobiose, D-turanose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, and potassium 2-ketogluconate are negative. Cells are susceptible to amoxicillin, ceftriaxone, imipenem, rifampicin, gentamicin, doxycline and vancomycin but resistant to ciprofloxacin, trimethoprim/sulfamethoxazole, erythromycin and metronidazole.

The G+C content of the genome is 67.2%. The 16S rRNA and genome sequences are deposited in GenBank under accession numbers HG726038 and CBYN00000000, respectively. The habitat of the microorganism is the human digestive tract. The type strain JCBT (= CSUR P778 = DSM 45997) was isolated from the fecal flora of a Saudi male who suffered from morbid obesity.

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References
1. Dubourg G, Lagier JC, Armougom F, Robert C, Hamad I, Brouqui P, Raoult D. The gut microbiota of a patient with resistant tuberculosis is more comprehensively studied by culturomics than by metagenomics. *Eur J Clin Microbiol Infect* 2013; 32:637-645. [PubMed](http://dx.doi.org/10.1007/s10096-012-1787-3)
2. Lagier JC, Armougom F, Millon M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G, Maraninchi M, et al. Microbial culuturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 2012; 18:1185-1193. [PubMed](http://dx.doi.org/10.1007/s10096-012-1787-3)
3. Tindall BJ, Rosselló-Móra R, Busse HJ, Ludwig W, Kämpfer 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/ijse.0.016949-0)
4. Database GOLD.
   http://www.genomesonline.org/cgi-bin/GOLD/index.cgi

5. Roux V, Million M, Robert C, Magne A, Raoult D. Non-contiguous finished genome sequence and description of Oceanobacillus massiliensis sp. nov. Stand Genomic Sci 2013; 9:370-384. http://dx.doi.org/10.4056/sigs.4267953

6. 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

7. 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. Gen. Nov. Stand Genomic Sci 2012; 370-384. PubMed http://dx.doi.org/10.4056/sigs.2415480

8. Mishra AK, Gimenez G, Lagier JC, Robert C, Raoult D, Fournier PE. Genome sequence and description of Alistipes senegalensis sp. nov. Gen. Nov. Stand Genomic Sci 2012; 51-116. PubMed http://dx.doi.org/10.4056/sigs.2956294

9. Lagier JC, Armougom F, Mishra AK, Nguyen TT, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Alistipes timonensis sp. nov. Gen. Nov. Stand Genomic Sci 2012; 315-324. PubMed http://dx.doi.org/10.4056/sigs.2685971

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

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

12. Mishra AK, Lagier JC, Rivet R, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Paenibacillus senegalensis sp. nov. Gen. Nov. Stand Genomic Sci 2012; 70-81. PubMed http://dx.doi.org/10.4056/sigs.3056450

13. Lagier JC, Gimenez G, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Herbaspirillum massiliense sp. nov. Gen. Nov. Stand Genomic Sci 2012; 200-209. PubMed

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

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

16. Ramasamy D, Kokcha S, Lagier JC, Nguyen TT, Raoult D, Fournier PE. Genomic sequence and description of Aeromicrobium massiliense sp. nov. Gen. Nov. Stand Genomic Sci 2012; 246-257. PubMed http://dx.doi.org/10.4056/sigs.3306717

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

18. Lagier JC, Elkarkouri K, Rivet R, Couderc C, Raoult D, Fournier PE. Genomic sequence and description of Senegalemassilia anaerobia gen. nov., sp. nov. Gen. Nov. Stand Genomic Sci 2013; 343-356. PubMed http://dx.doi.org/10.4056/sigs.3246665

19. Mishra AK, Hugon P, Lagier JC, Nguyen TT, Robert C, Couderc C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Peptoniphilus obesi sp. nov. Gen. Nov. Stand Genomic Sci 2013; 357-369. PubMed http://dx.doi.org/10.4056/sigs.32766871

20. Mishra AK, Lagier JC, Nguyen TT, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Peptoniphilus senegalensis sp. nov. Gen. Nov. Stand Genomic Sci 2013; 370-381. PubMed http://dx.doi.org/10.4056/sigs.3366764

21. Lagier JC, El Karkouri K, Mishra AK, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Enterobacter massiliensis sp. nov. Gen. Nov. Stand Genomic Sci 2013; 399-412. PubMed http://dx.doi.org/10.4056/sigs.3396830

22. Hugon P, Ramasamy D, Lagier JC, Rivet R, Couderc C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of Alistipes obesi sp. nov. Gen. Nov. Stand Genomic Sci 2013; 427-439. PubMed http://dx.doi.org/10.4056/sigs.3336746
23. Mishra AK, Hugon P, Robert C, Raoult D, Four- 
nier PE. Non contiguous-finished genome se- 
quence and description of Peptoniphilus 
grossensis sp. nov. Stand Genomic Sci 2012;  
7:320-330. PubMed

24. Mishra AK, Hugon P, Lagier JC, Nguyen TT, 
Couderc C, Raoult D, Fournier PE. Non contigu- 
ous-finished genome sequence and description of 
Enorma massiliensis gen. nov., sp. nov., a new 
member of the Family Coriobacteriaceae. Stand 
Genomic Sci 2013; 8:290-305. PubMed 
http://dx.doi.org/10.4056/sigs.3426906

25. Ramasamy D, Lagier JC, Gorlas A, Raoult D, 
Fournier PE. Non contiguous-finished genome se- 
quence and description of Bacillus 
massiloseneagelensis sp. nov. Stand Genomic Sci 
2013; 8:264-278. PubMed 
http://dx.doi.org/10.4056/sigs.3496989

26. Ramasamy D, Lagier JC, Nguyen TT, Raoult D, 
Fournier PE. Non contiguous-finished genome se- 
quence and description of Dielma fastidiosa gen. 
nov., sp. nov., a new member of the Family 
Erysi pelotrichaceae. Stand Genomic Sci 2013;  
8:336-351. PubMed 
http://dx.doi.org/10.4056/sigs.3567059

27. Mishra AK, Lagier JC, Robert C, Raoult D, Four- 
nier PE. Genome sequence and description of Timonella senegalensis gen. nov., sp. nov., a new 
member of the suborder Micrococccae. Stand 
Genomic Sci 2013; 8:318-335. PubMed 
http://dx.doi.org/10.4056/sigs.3476977

28. Mishra AK, Pfleiderer A, Lagier JC, Robert C, 
Raoult D, Fournier PE. Non contiguous-finished 
genome sequence and description of Bacillus 
massilanoarexius sp. nov. Stand Genomic Sci 
2013; 8:465-479. PubMed 
http://dx.doi.org/10.4056/sigs.4087826

29. Hugon P, Mishra AK, Lagier JC, Nguyen TT, 
Couderc C, Raoult D, Fournier PE. Non- 
contiguous finished genome sequence and de- 
scription of Brevibacillus massilensis sp. nov. 
Stand Genomic Sci 2013; 8:1-14. PubMed 
http://dx.doi.org/10.4056/sigs.346975

30. Hugon P, Mishra AK, Robert C, Raoult D, Four- 
nier PE. Non-contiguous finished genome se- 
quence and description of Anaerococcus 
vaginalis. Stand Genomic Sci 2012; 6:356-365. 
PubMed http://dx.doi.org/10.4056/sigs.2716452

31. Hugon P, Ramasamy D, Robert C, Couderc C, 
Raoult D, Fournier PE. Non-contiguous finished 
genome sequence and description of Kallipyga 
massiliensis gen. nov., sp. nov., a new member of 
the family Clostridiales Incertae Sedis XI. Stand 
Genomic Sci 2013; 8:500-515. PubMed 
http://dx.doi.org/10.4056/sigs.4047997

32. Padmanabhan R, Lagier JC, Dangui NPM, 
Michelle C, Couderc C, Raoult D, Fournier PE. 
Non-contiguous finished genome sequence and 
description of Megasphaera massiliensis. Stand 
Genomic Sci 2013; 8:525-538. PubMed 
http://dx.doi.org/10.4056/sigs.4077819

33. Mishra AK, Edouard S, Dangui NPM, Lagier JC, 
Caputo A, Blanch-Tailleur C, Ravaux I, Raoult D, 
Fournier PE. Non-contiguous finished genome se- 
quence and description of Nosocomicoccus 
massiliensis sp. nov. Stand Genomic Sci 2013; 
9:205-219. PubMed 
http://dx.doi.org/10.4056/sigs.4378121

34. Mishra AK, Lagier JC, Pfleiderer A, Nguyen TT, 
Caputo A, Raoult D, Fournier PE. Non-contiguous 
finished genome sequence and description of 
Holdemania massiliensis sp. nov. Stand Genomic 
Sci 2013; 9:395-409. 
http://dx.doi.org/10.4056/sigs.4628316

35. Ramasamy D, Mishra AK, Lagier JC, 
Padmanabhan R, Rossi-Tamisier M, Sentausa E, 
Raoult D, Fournier PE. A polyphasic strategy in- 
corporating genomic data for the taxonomic 
description of new bacterial species. Int J Syst 
Evol Microbiol 2014; (In press). PubMed 
http://dx.doi.org/10.1099/ijsem.0.057091-0

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

37. Collins MD, Smida J, Stackebrandt E. Phylogeneti- 
ic Evidence for the Transfer of Caseobacter 
polymorphus (Crombach) to the Genus 
Corynebacterium. Int J Syst Evol Microbiol 1989; 
39:7-9.

38. Aravena-Román M, Sproer C, Siering C, Inglis T, 
Schumann P, Yassin AF. Corynebacterium 
aquatimens sp. nov., a lipophilic 
Corynebacterium isolated from blood cultures of 
a patient with bacteremia. Syst Appl Microbiol 
2012; 35:380-384. PubMed 
http://dx.doi.org/10.1016/j.syapm.2012.06.008

39. Wagner KS, White JM, Lucenko I, Mercer D, 
Crowcroft NS, Neal S, Efstratiou A. Diphtheria in 
the postepidemic period, Europe, 2000-2009.
40. Bernard K. The genus corynebacterium and other medically relevant coryneform-like bacteria. J Clin Microbiol 2012; 50:3152-3158. PubMed http://dx.doi.org/10.1128/ICM.00796-12

41. 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

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

43. Garrity GM, Holt JG. The road map to the manual. In Bergey’s Manual® of Systematic Bacteriology 2011; 119-166.

44. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchical classification system, Actinobacteria classis nov. Int J Syst Bacteriol 1997; 47:479-491. http://dx.doi.org/10.1099/00207713-47-2-479

45. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. Int J Syst Bacteriol 1980; 30:225-420. http://dx.doi.org/10.1099/00207713-30-1-225

46. Zhi XY, Li WJ, Stackebrandt E. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. Int J Syst Evol Microbiol 2009; 59:589-608. PubMed http://dx.doi.org/10.1099/ijs.0.065780-0

47. Buchanan RE. Studies in the nomenclature and classification of bacteria. II. The primary subdivisions of the Schizomycetes. J Bacteriol 1917; 2:155-164. PubMed

48. Lehmann KB, Neumann R. Lehmann’s Medizin, Handatlannten. X Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik,. Fourth Edition, Volume 2, J.F. Lehmann, München, 1907, p. 270.

49. Bernard KA, Wiebe D, Burdz T, Reimer A, Ng B, Singh C, Schindle S, Pacheco AL. Assignment of Brevibacterium stations (ZoBell and Upaham 1944) Breed 1953 to the genus Corynebacterium, as Corynebacterium stations comb. nov., and emended description of the genus Corynebacterium to include isolates that can alkalinate citrate. Int J Syst Evol Microbiol 2010; 60:874-879. PubMed http://dx.doi.org/10.1099/ijs.0.012641-0

50. Lehmann KB, Neumann R. Atlas und Grundriss der Bakteriologie und Lehrbuch der speziellen bakteriologischen Diagnostik, First Edition, J.F. Lehmann, München, 1896, p. 1-448.

51. 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

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

53. 16S Youself database. (http://www.mediterrane-infection.com/article.php?iarub=152&tittre=16s-youself)

54. Riegel P, Ruym R, De Briel D, Prévost G, Jehl F, Christen R, Monteil H. Taxonomy of Corynebacterium diphtheriae and related taxa, with recognition of Corynebacterium ulcerans sp. nov. nom. rev. FEMS Microbiol Lett 1995; 126:271-276. PubMed http://dx.doi.org/10.1111/j.1574-6968.1995.tb07429.x

55. Fudou R, Jojima Y, Seto A, Yamada K, Kimura E, Nakamatsu T, Hiraishi A, Yamanaka S. Corynebacterium efficiens sp. nov., a glutamic-acid-producing species from soil and vegetables. Int J Syst Evol Microbiol 2002; 52:1127-1131. PubMed http://dx.doi.org/10.1099/ijs.0.02086-0

56. Kinoshita S, Takayama S, Akita S. Taxonomical study of glutamic acid accumulating bacteria, Micrococcus glutamicus sp. nov. Bull Agric Chem Soc Jpn 1958; 22:176-185. http://dx.doi.org/10.1271/bbb1924.22.176

57. Funke G, Hutson RA, Hilleringmann M, Heizmann WR, Collins MD. Corynebacterium lipophiloflavum sp. nov. isolated from a patient with bacterial vaginosis. FEMS Microbiol Lett 1997; 150:219-224. PubMed http://dx.doi.org/10.1016/S0378-1097(97)00118-3

58. Funke G, Ramos CP, Collins MD. Corynebacterium coyleae sp. nov., isolated from human clinical specimens. Int J Syst Bacteriol 1997; 47:92-96. PubMed http://dx.doi.org/10.1099/00207713-47-1-92

59. Yassin AF, Kroppenstedt RM, Ludwig W. Corynebacterium glaucum sp. nov. Int J Syst Evol Microbiol 2002; 52:1127-1131. PubMed http://dx.doi.org/10.1099/ijs.0.02086-0

Emerg Infect Dis 2012; 18:217-225. PubMed http://dx.doi.org/10.3201/eid1802.110987
Corynebacterium jeddahense

Microbiol 2003; 53:705-709. PubMed
http://dx.doi.org/10.1099/ijs.0.02394-0

60. Prodigal. http://prodigal.ornl.gov

61. Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res 2012; 40:D48-D53. PubMed
http://dx.doi.org/10.1093/nar/gkr1202

62. 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
http://dx.doi.org/10.1093/nar/25.5.0955

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

64. 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

65. 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

66. Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA, Barrell B. Artemis: sequence visualization and annotation. Bioinformatics 2000; 16:944-945. PubMed
http://dx.doi.org/10.1093/bioinformatics/16.10.944

67. Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J. DNA Plotter: circular and linear interactive genome visualization. Bioinformatics 2009; 25:119-120. PubMed
http://dx.doi.org/10.1093/bioinformatics/btn578

68. Darling AC, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 2004; 14:1394-1403. PubMed
http://dx.doi.org/10.1101/gr.2289704

69. Lechner M, Findeiss S, Steiner L, Marz M, Stadler PF, Prohaska SJ. Proteinortho: detection of (co-)orthologs in large-scale analysis. BMC Bioinformatics 2011; 12:124. PubMed
http://dx.doi.org/10.1186/1471-2105-12-124