Genome informatics and vaccine targets in *Corynebacterium urealyticum* using two whole genomes, comparative genomics, and reverse vaccinology

Luis Carlos Guimarães¹, Siomar de Castro Soares², Eva Trost³, Jochen Blom⁴, Rommel Thiago Jucá Ramos⁵, Artur Silva⁵, Debmalya Barh⁶, Vasco Azevedo¹*

*From X-meeting 2014 - International Conference on the Brazilian Association for Bioinformatics and Computational Biology
Belo Horizonte, Brazil. 28-30 October 2014*

**Abstract**

**Background:** *Corynebacterium urealyticum* is an opportunistic pathogen that normally lives on skin and mucous membranes in humans. This high Gram-positive bacteria can cause acute or encrusted cystitis, encrusted pyelitis, and pyelonephritis in immunocompromised patients. The bacteria is multi-drug resistant, and knowledge about the genes that contribute to its virulence is very limited. Two complete genome sequences were used in this comparative genomic study: *C. urealyticum* DSM 7109 and *C. urealyticum* DSM 7111.

**Results:** We used comparative genomics strategies to compare the two strains, DSM 7109 and DSM 7111, and to analyze their metabolic pathways, genome plasticity, and to predict putative antigenic targets. The genomes of these two strains together encode 2,115 non-redundant coding sequences, 1,823 of which are common to both genomes. We identified 188 strain-specific genes in DSM 7109 and 104 strain-specific genes in DSM 7111. The high number of strain-specific genes may be a result of horizontal gene transfer triggered by the large number of transposons in the genomes of these two strains. Screening for virulence factors revealed the presence of the *spaDEF* operon that encodes pili forming proteins. Therefore, *spaDEF* may play a pivotal role in facilitating the adhesion of the pathogen to the host tissue. Application of the reverse vaccinology method revealed 19 putative antigenic proteins that may be used in future studies as candidate drug or vaccine targets.

**Conclusions:** The genome features and the presence of virulence factors in genomic islands in the two strains of *C. urealyticum* provide insights in the lifestyle of this opportunistic pathogen and may be useful in developing future therapeutic strategies.

---

**Background**

The species *Corynebacterium urealyticum* was proposed in 1986, but this bacteria was first isolated between June 1983 and March 1984, when four patients were diagnosed with alkaline-encrusted cystitis. The published case report describes the isolates as belonging to the Corynebacterium group D2 [1,2]. Chemotaxonomic studies and 16S rRNA sequence comparisons showed that *C. urealyticum* was more closely related to Corynebacterium lipophilic species such as *Corynebacterium jeikeium*, but could be differentiated from *C. jeikeium* based on its ability to hydrolyze urea [3]. *C. urealyticum* is a Gram-positive, non-spore-forming, aerobic, and slow-growing bacteria. Its cell wall is composed of peptidoglycan, menaquinone, mycolic acids, and cellular fatty
acids, which is the common composition of the cell walls of Corynebacterium species [4].

C. urealyticum is an opportunistic pathogen commonly isolated from the skin and mucous membranes of hospitalized patients. The pathogen mainly causes acute or encrusted cystitis, encrusted pyelitis, and pyelonephritis [4]. Its urease activity is the main factor that contributes to the ability of C. urealyticum to colonize the urinary tract where its presence is associated with alkaline pH and the formation of ammonium magnesium phosphate stones [1,2]. C. urealyticum is a multi-drug resistant bacterium and its treatment requires the administration of multiple drugs and additional invasive interventions [4,5]. Currently, the complete genome sequences of only two C. urealyticum strains, DSM 7109 and DSM 7111, are publicly available. In these two genomes, the antibiotic resistance genes were located in mobile DNA, suggesting that the multidrug resistance was acquired through horizontal gene transfer [6,7].

In this work, we compared the genome sequences of the two C. urealyticum multidrug resistance strains DSM 7109 and DSM 7111, focusing on differences in the gene content and metabolic pathways between the two strains. We also attempted to identify new candidate targets that can be used in the development of drugs or vaccines against this pathogen.

Methods
Genome sequences of C. urealyticum strains DSM 7109 and DSM 7111

C. urealyticum DSM 7109 was isolated from a patient with alkaline-encrusted cystitis and C. urealyticum DSM 7111 was isolated from the urine samples of a 9-year-old patient with an ectopic kidney. The genome sequences of both these strains were retrieved from the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) [8] [GenBank:NC_010545.1, GenBank: NC_020230.1].

Bioinformatics analysis

The origin of chromosomal DNA replication (oriC) gene was predicted using the Ori-Finder web program [9]. The Ori-Finder prediction was based on a combined process: (i) gene identification involving analysis of base composition through Z-curve method; and, (ii) occurrence of genes frequently close to oriCs (distribution of dnaA boxes along the genome) [9]. The CRISPRs were predicted using the CRISPRfinder web program [10]. These regions are important because they confer protection against bacteriophages. The comparative analysis was done using the EDGAR web-program that compares genome content based on the calculation of the BLAST score ratio by automatically adjusted cutoff for each selected dataset [11].

In silico identification metabolic pathway construction

The metabolic pathways reconstruction of C. urealyticum was performed using the genome sequence file in FASTA format and the genome annotation file in GBK format. Metabolic pathways databases for strains DSM 7109 and DSM 7111 were created using the Pathway Tools 13 software (available at http://bioinformatics.ai.sri.com/ptools/), developed by SRI International [12]. The Pathway Tools software contains algorithms that can predict the metabolic pathways of an organism from its genome by comparing it to a reference pathways database known as the MetaCyc Database [13]. Construction of a metabolic pathways database was done using the BioCyc collection [14].

Prediction of genome plasticity of C. urealyticum DSM 7109 and DSM 7111 strains

Prediction of genomic islands in C. urealyticum genomes was done using PIPS software [15]. PIPS detects genome signatures like C+G content, codon usage deviation, high concentrations of virulence factors, hypothetical proteins, the presence of transposases and tRNA flanking sequences, and absence of query regions in non-pathogenic organisms. C. glutamicum ATCC 13032 was used as the closely related non-pathogenic species to C. urealyticum in PIPS. The BRIG software [16] was used for plasticity comparisons among C. urealyticum (DSM 7109 and DSM 7111 strains), C. pseudotuberculosis 1002, C. diphtheriae NCTC 13129, C. ulcerans 809, and C. glutamicum ATCC 13032.

Prediction of putative antigenic targets of C. urealyticum

To identify antigenic targets, we used the strategy described by Barh et al. [17] with modifications. We also adopted the four rules as per the reverse vaccinology strategy of Rappuoli et al. [18] for final selection of the putative vaccine targets. Rule I: consider the antigenic proteins that are either secreted proteins, surface-exposed proteins, or membrane proteins so that they can be exposed to the host, and therefore can be promptly recognized by the host immune system [18]; Rule II: major histocompatibility complex (MHC) I and II binding properties with adhesion probability greater than 0.51 and absence of similarity to host proteins [19]; Rule III: protein conservation among different genomes [19]; and Rule IV: virulence factors are normally encoded within genomic islands [18]. Rule IV does not exclude the targets from Rule III.

SurfG+ software [20] was used to predict targets according to the Rule I. This software classifies proteins according to their subcellular location using the presence or absence of signal peptides, retention signals, and transmembrane helices. To apply Rule II, the
proteins predicted by surfG+ were analyzed using the Vaxign software [19]. Because the aim of this work was to identify vaccine candidates, the predicted proteomes were screened for proteins that were potentially antigenic in both strains (Rule III). To achieve this, we used the Artemis Comparison Tool [21] with BLAST alignment comparison files and searched for antigenic proteins that show more than 70% similarity in 70% of their extensions in both strains. Base on Rule IV, we screened the detected antigenic proteins for antigenic targets harbored by shared genomic islands in the two strains.

**Results and discussion**

**Genomic architecture and features of C. urealyticum strains DSM 7109 and DSM7111**

Strains DSM 7109 and DSM 7111 were isolated from patients with alkaline-encrusted cystitis [6,7]. The genomic composition of these two strains is very similar; i.e., both sequences have the same G+C content, coding density, ribosomal RNAs clusters, and clustered regularly interspaced short palindromic repeats (CRISPRs). However, the sizes of the two genomes are different: the DSM 7111 genome contains 2,316,065 bp and is 50 Kb smaller than the DSM 7109 genome with 2,369,219 bp. As expected, the number of coding sequences in the DSM 7111 genome (1,927 protein coding regions) is lower than in the DSM 7109 genome (2,011 protein coding regions) because both genomes have the same coding density. These data indicate a strain-specific difference in the gene repertoires in both these isolates. Relevant data and general features from both genome sequences are summarized in Table 1.

Our analysis of the GC skew [(G-C)/(G+C)] revealed that both genome sequences contained a bi-directional replication mechanism (Figure 1). The origin of the chromosomal replication (oriC) gene is located between the replication initiator genes dnaA (downstream) and dnaN (upstream) and has a size of 843 bp. However, the G/C skew analysis did not confirm that the replication termination site, dif, was located at the 180° position from oriC [9,22].

CRISPRs are often associated with cas genes that normally provide resistance against bacteriophages [23]. One CRISPR region was predicted in each genome (strains DSM 7109 and DSM 7111) using the CRISPRFinder software [10]. Both these regions were flanked by seven cas genes. The size of one CRISPR was 28 bp and the consensus sequence was the same for the CRISPRs in both genomes. Each CRISPR was separated by 69 bp (Table 2).

**Gene sharing among the two C. urealyticum strains**

Orthologous genes were detected using the EDGAR software, which defines subsets of genes using the SRV method to predict orthologous genes in prokaryotic genomes [11]. We found that the DSM 7109 and DSM 7111 genomes together encode 2,115 no-redundant coding gene sequences; 1,823 (86.2%) of these coding gene sequences were common to both strains, and 188 and 104 were specific to DSM 7109 and DSM 7111, respectively. Species-specific genes have been linked to niche adaptation of microorganisms. A previous study of 17 *Escherichia coli* strains found that less than 50% of the genes (2,200 genes in a total of 5,000 genes) were shared among these strains [24]. Therefore, we can infer that the DSM 7109 and DSM 7111 genomes are very similar because they share a high proportion of their genes. A previous study of four *Corynebacterium pseudotuberculosis* strains [25] also reported a large number in shared genes; 1,851 (77.9%) genes in a total of 2,377 genes. Clearly, the numbers of core genes are likely to reduce when more strains of *C. urealyticum* are added, as was shown previously by Soares and colleagues in a study of 15 *C. pseudotuberculosis* strains were 1,504 (54.5%) genes in a total of 2,782 genes were shared [26].

**Plasticity of the C. urealyticum DSM 7109 and DSM 7111 genomes**

Genome plasticity has been used to provide insights into genome evolution through the study of horizontally acquired genomic regions. The transfer of blocks of genes (genomic islands) normally correlates with the acquirement of a given function, like virulence (pathogenicity islands), degradation of secondary compounds (metabolic islands), antibiotic resistance (resistance islands) and symbiotic relationships with Leguminosae (symbiotic islands) [15]. Furthermore, because genomic islands are acquired from a different organism, they are responsible for deviations in genomic signatures such as codon usage and G+C content once they reflect the genomic signature of the donor organism [27]. We used the PIPS software [15] to predict genomic islands in both *C. urealyticum* strains. Twenty-six genomic islands were predicted in each genome (Figure 1). DSM 7109 had 556 genes present in the genomic islands and DSM 7111 had 496 genes. We identified 403 genes in the genomic islands that belonged to the shared genes.

| Feature | DSM 7109 | DSM 7111 |
|---------|----------|----------|
| Genome size (bp) | 2,369,219 | 2,316,065 |
| C+G content (%) | 64.2 | 64.2 |
| Coding sequences | 2,084 | 2,007 |
| Coding density (%) | 90.16 | 89.53 |
| Ribosomal RNAs | 3 × (16S - 23S - 5S) | 3 × (16S - 23S - 5S) |
| Transfer RNAs | 51 | 54 |
dataset, meaning that the majority of genes that were acquired by horizontal gene transfer were commonly shared genes.

Previous studies on genomic islands in *C. pseudotuberculosis* and *C. diphtheriae* identified 16 and 52 genomic islands, respectively [26,28]. Only nine of the genes in the genomic islands were shared among *C. urealyticum* strains DSM 7109 and DSM 7111, *C. pseudotuberculosis* 1002, and *C. diphtheriae* NCTC 13129 (Table 3). The low number of shared genes among different species in the same genus is expected because their habitats are different and genomic islands are normally acquired through horizontal gene transfer as was shown previously by Perrin and colleagues [29]. Pathogenicity islands contain genes correlated with virulence [27]. Therefore, these genes may be good candidates for the development of vaccines or drugs [15]. When we compared the orthologs of the candidate virulence factors reported in *C. ulcerans* [30] with the genes in the pathogenicity islands in *C. urealyticum* we found only one common gene and this gene was annotated with unknown function (ID: CU7111_1212 for DSM 7111; ID: cur_1230 for DSM 7109).

**Figure 1** Comparative genomic maps of *C. urealyticum* strains DSM 7109 and DSM 7111. The *C. urealyticum* DSM 7109 map (left) used as the reference. The *C. urealyticum* DSM 7111 map (right) used as the reference. From the inner to outer circles on the genome maps: *C. urealyticum* DSM 7109 (Cur_7109) then *C. urealyticum* DSM 7111 (Cur_7111) for the map on the left; *C. urealyticum* DSM 7111 (Cur_7111) then *C. urealyticum* DSM 7109 (Cur_7109) for the map on the right, followed by *C. pseudotuberculosis* 1002 (Cp_1002), *C. diphtheriae* NCTC 13129 (Cd_NCTC13129), *C. ulcerans* 809 (Cul_809), *C. glutamicum* ATCC 13032 (Cg_ATCC13032). The outermost circles in both maps indicate the 26 genomic islands (PiCur 1-26).

### Table 2. Structural features of CRISPR loci predicted in *C. urealyticum* strains DSM 7109 and DSM 7111

| Strain                       | No. of CRISPR loci | No. of cas genes | locus_tag cas genes | No. of spacers | CRISPR size | *CRISPR consensus sequence         |
|------------------------------|--------------------|------------------|---------------------|----------------|-------------|------------------------------------|
| *Corynebacterium urealyticum* DSM 7109 | 1                  | 7                | cur_1967 cur_1968 cur_1969 cur_1970 cur_1971 cur_1972 cur_1973 | 69             | 28 bp                   | GGCTCATCCCCGCTGGCGGGGAGCAC         |
| *Corynebacterium urealyticum* DSM 7111 | 1                  | 7                | CU7111_1887 CU7111_1888 CU7111_1889 CU7111_1890 CU7111_1891 CU7111_1892 CU7111_1893 | 69             | 28 bp                   | GGCTCATCCCCGCTGGCGGGGAGCAC         |

*CRISPR, clustered regularly interspaced short palindromic repeats*
The sub-cellular location of proteins in DSM 7109 and DSM 7111 was predicted using the SurfG+ software [31], which classifies genes into four categories: cytoplasmic, membrane, PSE (putative surface-exposed), and secreted (Table 4). We used the four rules described in the reverse vaccinology strategy (see the Methods section for details) for final selection of putative vaccine targets. According to Rule I, proteins exposed to the host are better candidates because they can be promptly recognized by the immune system; for example, secreted proteins, surface-exposed proteins, and membrane proteins. We predicted 590 and 579 putative candidates for DSM 7109 and DSM 7111, respectively, using Rule I. The encoded proteins were submitted to the Vaxign software [19], which detected 54 and 57 proteins with antigenic properties in DSM 7109 and DSM 7111, respectively. Using Rule III, we considered only proteins that were shared by both strains, which resulted in 46 candidates for both strains. Finally, using Rule IV, we identified 19 proteins that were shared by both strains and that were encoded within genomic islands as vaccine candidates (Table 5). Among these 19 vaccine candidates, six were annotated with a function and a gene name. These proteins have been identified as potential vaccine targets in previous studies [30,32-36], but as yet no tests have been carried out to confirm this.

Table 3. Genes present in genomic islands shared by related Corynebacterium species

| Gene        | C. urealyticum DSM 7109 | C. urealyticum DSM 7111 | C. pseudotuberculosis 1002 | C. diphtheriae NCTC 13129 | Product                        |
|-------------|-------------------------|-------------------------|-----------------------------|---------------------------|--------------------------------|
| cur_1756    | CU7111_1693             | Cp1002_1932             | DIP2331                     | -                         | Putative aldehyde dehydrogenase |
| cur_1817    | CU7111_1751             | Cp1002_1909             | DIP2133                     | -                         | Fe-S oxidoreductase             |
| cur_1897    | CU7111_1828             | Cp1002_1870             | DIP0236                     | srtB                      | Fimbrial associated sortase (Surface protein transpeptidase) |
| cur_1898    | CU7111_1829             | Cp1002_1872             | DIP0235                     | spaD                      | Putative surface-anchored protein (Fimbrial subunit) |
| cur_1899    | CU7111_1830             | Cp1002_1874             | DIP0233                     | srtC                      | Fimbrial associated sortase (Surface protein transpeptidase) |
| cur_1933    | CU7111_1856             | Cp1002_0132             | DIP0247                     | tadA                      | tRNA-specific adenosine deaminase |
| cur_1934    | CU7111_1857             | Cp1002_0131             | DIP0246                     | -                         | Hypothetical protein           |
| cur_1935    | CU7111_1858             | Cp1002_0130             | DIP0245                     | tynA                      | Prephenate dehydrogenase       |
| cur_1939    | CU7111_1861             | Cp1002_0120             | DIP0179                     | -                         | Putative dicarboxylate uptake system |

The genomes of C. urealyticum strains DSM 7109 and DSM 7111, C. pseudotuberculosis 1002, and C. diphtheriae NCTC 13129 were compared.

The rpfC gene (resuscitation-promoting factor) is a member of a protein family (rpfA, rpfB, rpfD, and rpfE) found in Actinobacteria. The protein encoded by rpfC plays a role in stimulating resuscitation of dormant cells and in the multiplication of normal viable bacteria. Studies in Mycobacterium luteus with a disrupted rpf gene were not possible because of the absence of a second functional copy of the gene, suggesting that this protein is essential for normal growth and reduces the lag phase of diluted fast-growers [32,37].

The mepA gene (penicillin-insensitive murein endopeptidase) in E. coli encodes a protein the cleaves the D-alanyl-meso-2,6-diamino-pimelyl amide bond of peptidoglycans [38]; however, this protein is sensitive to metal-chelating agents such as lipoteichoic acid and deoxyribonucleic acid [39]. Previous studies with metallopeptidases showed that animals infected with C. pseudotuberculosis reacted to the mepA protein, while non-infected animals did not. This protein has transmembrane domains, another strong indication that it may make a good molecular vaccine target [33,40].

The lpps (lipoprotein) gene encodes a protein that is associated with cell envelopes and has four known lipoprotein functions: (i) structural function (murein lipoproteins); (ii) transport function (substrate-binding proteins of ABC transporters in Gram-positive bacteria); (iii) adhesion function; and (iv) enzymatic function. The lipoprotein present in C. urealyticum has an L,D-transpeptidase catalytic domain, which gives the bacteria the ability to resist beta-lactam antibiotics by inhibiting PBPs (penicillin-binding protein) [34,41].

The cmntA (trehalose corynomycol transferase) gene encodes a protein that has catalytic function. It plays a role in the transfer of mycolic acids through trehalose monocorynomycolate on the cell wall arabinogalactan to
another trehalose monocorynomycolate to produce trehalose dicorynomycolate [42].

The \textit{spaD} and \textit{spaE} genes are part of the \textit{spaDEF} cluster that encodes adhesive pilus structures that are surface-anchored to the cell walls of \textit{Corynebacterium} where they probably facilitate the adhesion of the pathogen to the host tissue. We detected the \textit{spaF} gene in the DSM 7109 and DSM 7111 genomes, as well as the sortase encoding genes \textit{srtB} and \textit{srtC}. The genome organization of the \textit{spaDEF} cluster in the two \textit{C. urealyticum} strains is similar to cluster organization in \textit{C. diphtheriae} NCTC 13129 and \textit{C. ulcerans} 809 and BR-AD22 strains [30,43]. However, the \textit{spaABC} cluster of genes proposed as an essential virulence factor in \textit{C. diphtheria} [35] was absent in both strains of \textit{C. urealyticum} analyzed.

The \textit{crcB} gene encodes a putative membrane protein, important for the reducing the fluoride concentration in cells, thus reducing its toxicity. Fluoride ions reduce cell growth, even when present in millimolar concentrations. Thus, we can infer that \textit{crcB} gene is an efficient resistance mechanism [36].

### Differences in metabolic pathways in the \textit{C. urealyticum} genomes

To predict the metabolic pathways encoded in the DSM 7109 and DSM 7111 genomes, we used the Pathway Tools software (version 13.0) [44], and detected 226 and 271 pathways in DSM 7109 and DSM 7111, respectively. We also identified 942 and 1,116 metabolic reactions for these strains (Table 6).

### Comparative analysis of two pathway classes (Bio-synthesis and Degradation/Utilization/Assimilation)

Comparative analysis of two pathway classes (Bio-synthesis and Degradation/Utilization/Assimilation) showed that the DSM 7109 and DSM 7111 genomes had 139 and 174 Biosynthesis pathways, respectively (Table 7), which is quite different from the number of pathways we found previously in other species in the same genus; for example, \textit{C. pseudotuberculosis} strains 1002 and C231 in which 105 and 104 Biosynthesis pathways were predicted, respectively [25]. The number of Degradation/Utilization/Assimilation pathways predicted in the DSM 7109 and DSM 7111 genomes where similar, 70 pathways in DSM 7109 and 66 pathways in DSM 7111 (Table 7).

On further analysis, we found that the DSM 7109 and DSM 7111 genomes had 25 and 57 unique metabolic pathways (Table S1, additional file 1), respectively, even though both the strains were isolated from humans and caused the same symptoms [6,7].

### Table 5. Putative antigenic proteins identified using Vaxign and shared by two \textit{C. urealyticum} strains

| DSM 7109 - Locus_Tag | DSM 7111 - Locus_Tag | Gene name | Subcellular location | Gene product                  |
|---------------------|----------------------|-----------|----------------------|-------------------------------|
| cur_0025            | CU7111_0027          | \textit{rpfC} | secreted             | Resuscitation-promoting factor RpfC |
| cur_0151            | CU7111_0157          | -         | secreted             | Putative secreted protein     |
| cur_0291            | CU7111_0284          | -         | PSE                  | Putative surface-anchored protein |
| cur_0295            | CU7111_0288          | -         | PSE                  | Hypothetical protein          |
| cur_0527            | CU7111_0510          | -         | PSE                  | Putative secreted protein     |
| cur_0530            | CU7111_0513          | \textit{mepA} | secreted             | Putative secreted metallopeptidase |
| cur_1309            | CU7111_1290          | -         | PSE                  | Hypothetical protein          |
| cur_1319            | CU7111_1300          | -         | PSE                  | Putative ribonuclease         |
| cur_1350            | CU7111_1330          | -         | secreted             | Putative secreted protein     |
| cur_1399            | CU7111_1390          | \textit{lppS} | PSE                  | Putative lipoprotein          |
| cur_1604            | CU7111_1545          | -         | secreted             | Hypothetical protein          |
| cur_1636            | CU7111_1577          | -         | secreted             | Putative secreted protein     |
| cur_1834            | CU7111_1766          | -         | PSE                  | Hypothetical protein          |
| cur_1842            | CU7111_1775          | \textit{cmtA} | secreted             | Trehalose corynomycolyl transferase A |
| cur_1896            | CU7111_1827          | \textit{spaE} | PSE                  | Putative surface-anchored protein (fimbrial subunit) |
| cur_1898            | CU7111_1829          | \textit{spaO} | PSE                  | Putative surface-anchored protein (fimbrial subunit) |
| cur_1958            | CU7111_1880          | -         | secreted             | Hypothetical protein          |
| cur_1959            | CU7111_1881          | -         | secreted             | Hypothetical protein          |
| cur_1980            | CU7111_1900          | \textit{crcB} | membrane             | Putative fluoride ion transporter CrcB |

### Table 6. Numbers of gene data types in \textit{C. urealyticum} strains DSM 7109 and DSM 7111

| Data type          | DSM 7109 | DSM 7111 |
|--------------------|----------|----------|
| Genes              | 2082     | 1998     |
| Pathways           | 226      | 271      |
| Metabolic reactions| 942      | 1116     |
| Transport reactions | 34       | 36       |
| Polypeptides       | 2022     | 1935     |
| Enzymes            | 536      | 543      |
Conclusions
To our knowledge, this is the first comparative genomic study using the complete genome sequences of two *C. urealyticum* strains, DSM 7109 and DSM 7111. Our analyses provided insights into the genome architecture and the gene content of this species. We found that the *C. urealyticum* DSM 7111 genome was 50 kb shorter than the *C. urealyticum* DSM 7109 genome. This difference in genome size may be linked to the large number of genomic islands (26 for each genome) predicted for both genomes. The genomic islands may have resulted from the horizontal transfer of genes, leading to the acquisition of many strain-specific genes. We detected a high number of strain-specific genes in the two genomes compared with the low number of species-specific genes that have been reported in previous studies of other species of Corynebacterium [25,30]. The horizontal transfer of genes may also explain why *C. urealyticum* is multidrug resistant; i.e., it has received virulence genes by horizontal transfer [4,5].

*C. urealyticum* is a pathogenic opportunistic bacteria although it showed the *spaDEF* operon (virulence factor), with a structure similar to that of pathogenic species like *C. diphtheriae* and *C. ulcerans*. This operon encodes an adhesive pilus responsible for facilitating the adhesion of the pathogen to host cells [28,43].

This comparative genomic study of two *C. urealyticum* strains provides a basis using reverse vaccinology to predict new antigenic targets. However, additional *C. urealyticum* strains will have to be studied to create effective vaccines against this bacterium.

Additional material

Table 7. Numbers of pathways in *C. urealyticum* strains DSM 7109 and DSM 7111

| Pathway class | DSM 7109 | DSM 7111 |
|---------------|----------|----------|
| **Biosynthesis** |          |          |
| - Amines and Polyamines Biosynthesis | 4 | 5 |
| - Amino Acids Biosynthesis | 27 | 32 |
| - Aminoacyl-tRNA Charging | 3 | 3 |
| - Aromatic Compounds Biosynthesis | 2 | 4 |
| - Carbohydrates Biosynthesis | 6 | 9 |
| - Cell Structures Biosynthesis | 4 | 5 |
| - Cofactors, Prosthetic Groups, Electron Carriers Biosynthesis | 34 | 40 |
| - Fatty Acids and Lipids Biosynthesis | 6 | 8 |
| - Metabolic Regulators Biosynthesis | 1 | 1 |
| - Nucleosides and Nucleotides Biosynthesis | 18 | 25 |
| - Other Biosynthesis | 5 | 5 |
| - Secondary Metabolites Biosynthesis | 4 | 3 |
| **Degradation/Utilization/Assimilation** |          |          |
| - Alcohols Degradation | 1 | 4 |
| - Amines and Polyamines Degradation | 5 | 3 |
| - Amino Acids Degradation | 18 | 17 |
| - Aromatic Compounds Degradation | 3 | 2 |
| - Cl Compounds Utilization and Assimilation | 1 | 3 |
| - Carbohydrates Degradation | 4 | 2 |
| - Carboxylates Degradation | 5 | 6 |
| - Degradation/Utilization/Assimilation - Other | 2 | 1 |
| - Fatty Acid and Lipids Degradation | 3 | 4 |
| - Inorganic Nutrients Metabolism | 10 | 8 |
| - Nucleosides and Nucleotides Degradation | 6 | 5 |
| - Protein Degradation | 2 | 2 |
| - Secondary Metabolites Degradation | 2 | 2 |
| - Steroids Degradation | 2 | 2 |
| **Generation of Precursor Metabolites and Energy** |          |          |
| - Total | 185 | 216 |

Additional file 1: Table S1 *C. urealyticum* strain-specific pathways.

Competing interests
The authors declare that they have no competing interests.
Authors' contributions
LCG, SCS, and VA conceived the study and designed the experiments. LCG, SCS, ET, and RTRJ performed the experiments. LCG, SCS, and TRJR analyzed the data. SCS, JB, AS, DB, and VA contributed the materials and analyzes tools. AS, DB, JB, and VA provided insights and technical inputs. LCG wrote the paper and SCS, ET, and DB edited the manuscript. All authors read and approved the final manuscript.

Acknowledgements
We thank the CAPES/PDSE International Cooperation for financing an exchange scholarship (http://www.capes.gov.br/bio coopera çao internacional) (grant number 9151125) and Graduate Cluster Industrial Biotechnology (CLUB, http://www.graduatedcluster.net/) for support this research.

Declarations
The publication of this work is funded by CNPq (National Counsel of Technological and Scientific Development), FAPESP (Foundation for Research Support of the State of Minas Gerais) and CAPES (Coordination for the Improvement of Higher Education Personnel) number SC-1000675. This article has been published as part of BMC Genomics Volume 16 Supplement 5, 2015: Proceedings of the 10th International Conference of the Brazilian Association for Bioinformatics and Computational Biology (X-Meeting 2014). The full contents of the supplement are available online at http://www.biomedcentral.com/biome dici nalsupplements/16/55.

Authors' details
1 Department of General Biology, Institute of Biological Sciences, Federal University of Minas Gerais, Avenue Antônio Carlos, 6627, Belo Horizonte, Minas Gerais, Brazil. 2 Department of Preventive Veterinary Medicine, School of Veterinary Medicine, Federal University of Minas Gerais, Avenue Antônio Carlos, 6627, Belo Horizonte, Minas Gerais, Brazil. 3 Institut für Hygiene, Universitätssanitätsklinikum Münster, Albert-Schweitzer-Campus 1, Münster, Germany. 4 Bioinformatics and Systems Biology, Justus-Liebig-University Giessen, Ludwigstasse, 23, Giessen, Germany. 5 Department of Genetics, Institute of Integrative Omics and Applied Biotechnology (IIOAB), Nonakari, Pumba Medindupur, WB-721172, India.

Published: 26 May 2015

References
1. Soriano F, Ponte C, Santamaría M, Aguado JM, Wilhelm I, Vela R, Delatte LC: Corynebacterium group D2 as a cause of alkaline-encrusted cystitis: report of four cases and characterization of the organisms. J Clin Microbiol 1985, 21(5):788-792.
2. Soriano F, Ponte C, Santamaria M, Castillo C, Fernández Roblas R: In vitro and in vivo study of stone formation by Corynebacterium group D2 (Corynebacterium urealyticum). J Microbiol Methods 1996, 23(4):691-694.
3. Pitcher D, Soto a, Soriano F, Valero-Guillem P: Classification of coryneform bacteria associated with human urinary tract infection (group D2) as Corynebacterium urealyticum sp. nov. Int J Syst Bacteriol 1992, 42(1):178-181.
4. Soriano F, Tauch A: Microbiological and clinical features of Corynebacterium urealyticum: urinary tract stones and genomics as the Rosetta Stone. Clin Microbiol Infect 2008, 14(T1):632-643.
5. García-Bravo M, Aguado JM, Morales JM, Noriega AR: Evidence for external factors in resistance of Corynebacterium urealyticum to antimicrobial agents. Antimicrob Agents Chemother 1996, 40(2):497-499.
6. Tauch A, Trost E, Tilk er A, Luxberger U, Schreier S, Goessmann A, et al: The Lifestyle of Corynebacterium urealyticum derived from its complete genome sequence established by pyrosequencing. J Bacteriol 2008, 190(2):11-21.
7. Guimarães LC, Soares SC, Albersmeier A, Blom J, Jaenicke S, Azevedo V, et al: Complete Genome Sequence of Corynebacterium urealyticum Strain DSM 7111, Isolated from a 9-Year-Old Patient with Alkaline. J Bacteriol 2013, 1(3):2008-2009.
8. Benson D a, Haas S, Rutschkow A a, Lipman DJ, Ostell J, Wheeler DL: GenBank. Nucleic Acids Res 2005, 33(Database issue):D34-D38.
9. Gao F, Zhang C-T: Ori-Finder: a web-based system for finding oris in unannotated bacterial genomes. BMC Bioinformatics 2008, 9:79.
10. Grissa I, Vergnaud G, Pourcel C: CRISPFind: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 2007, 35(Web Server issue):W52-W57.
11. Blom J, Albaugh SP, Doppmeier D, Puhlcr H, Vahalier F, Zakrzewski M, Goessmann A: EDGAR: a software framework for the comparative analysis of prokaryotic genomes. BMC Bioinformatics 2009, 10:154.
12. Karp PD, Paley S, Romero P: The Pathway Tools software. Bioinformatics 2002, 18(Suppl 1):S22-S23.
13. Casia R, Attman T, Dreher K, Fulcher CA, Subhraveti P, Keseler IM, et al: The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. Nucleic Acids Res 2012, 40(Database issue):D742-D753.
14. Casia R, Karp PD: Using the MetaCyc Pathway Database and the BioCyc Database Collection 2007, Chapter 1(December). Unit1:17.
15. Soares SC, Abreu VA, Ramos RT, Cereida L, Silva A, Baumbach J, et al: PIPS: pathogenicity island prediction software. PLoS One 2012, 7(2):e35848.
16. Alkilain NF, Petty NK, Ben Zakour NL, Beasiant SA: BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 2011, 12:402.
17. Bah D, Misra AN, Kumar A, Vasco A: A novel strategy of epitope design in Neisseria gonorrhoeae. Bioinformat 2010, 5(2):77-85.
18. Rappoporti R: Reverse vaccinology: a genome-based approach for vaccine development. Vaccine 2001, 19:17-19: 2688-2690.
19. He Y, Xiang Z, Mobley HL: Vaxinf: the first web-based vaccine design program for reverse vaccinology and applications for vaccine development. J Biomed Biotechnol 2010, 2012:297505.
20. Barnov A, Loux V, Hammarri A, Nicolas P, Langella P, Ehrlich D, et al: Prediction of surface exposed proteins in Streptococcus pyogenes, with a potential application to other Gram-positive bacteria. Proteomics 2009, 9(11):61-173.
21. Caner TJ, Rutherford KM, Beriman M, Rajandream MA, Barrell BG, Parkhill J: ACT: the Artemis Comparison Tool. Bioinformatics 2005, 21(16):3422-3423.
22. Hendrickson H, Lawrence JG: Mutational bias suggests that replication termination occurs near the dif site, not at Ter sites. Mol Microbiol 2007, 64(1):42-56.
23. Devaiah H, Garneau JE, Moineau S: CRISPR/Cas system and its role in phase-bacteria interactions. Annu Rev Microbiol 2010, 64:475-493.
24. Raiko Da, Rosowitz MJ, Myers GS, Mongodin EF, Fricke WF, Gajer P, et al: The pangenome structure of Escherichia coli: comparative genomic analysis of E. coli commensal and pathogenic isolates. J Bacteriol 2008, 190:6881-6893.
25. Ruiz JC, DAconsseca V, Silva A, Ali A, Pinto AC, Santos AR, et al: Evidence for reductive genome evolution and lateral acquisition of virulence functions in two Corynebacterium pseudotuberculosis strains. PLoS One 2011, 6(4):e18551.
26. Soares SC, Silva A, Trost E, Blom J, Ramos R, Carneiro A, et al: The Pan-Genome of the Animal Pathogen Corynebacterium pseudotuberculosis Reveals Differences in Genome Plasticity between the Biovar ovis and equi Strains. PLoS One 2013, 8(1):e53818.
27. Schmidt H, Hensel M: Pathogenicity Islands in Bacterial Pathogenesis. Pathogenicity Islands in Bacterial Pathogenesis. 2004, 17(1):14-56.
28. Trost E, Blom J, Soares SDC, Huang IH, Al-Dilami A, Schröder J, et al: The Pathogenicity Islands of Corynebacterium diphtheriae that provides insights into the genomic diversity of pathogenic isolates from cases of classical diphtheria, endocarditis, and pneumonia. J Bacteriol 2012, 194(12):3199-3215.
29. Perin A, Bonacorsi S, Carbonelle E, Talibi D, Deissen P, Nassif X, Tinsley C: Comparative Genomics Identifies the Genetic Islands That Distinguish Neisseria meningitidis, the Agent of Cerebrospinal Meningitis, from Other Neisseria Species Comparative Genomics Identifies the Genetic Islands That Distinguish Neisseria meningitidis. 2002, 70(12):7063-7072.
30. Trost E, Al-Dilami A, Papavasiliou P, Schneider J, Vliehove P, Burkovski A, et al: Comparative analysis of two complete Corynebacterium ulcerans genomes and detection of candidate virulence factors. BMC Genomics 2011, 12:383.
31. Santos AR, Pereira VB, Barbosa E, Baumbach J, Pinto AC, Santos AR, et al: Pathogenicity Islands in Bacterial Pathogenesis. Pathogenicity Islands in Bacterial Pathogenesis. 2008, 6(4):611-621.
33. Seyffert N. Isolamento, identificação e análise molecular do gene cpsec39 codificador de uma proteína exclusiva de Corynebacterium pseudotuberculosis: perspectivas no desenvolvimento de métodos profiláticos para o controle da linfadenite caseosa. Federal University of Minas Gerais 2009.

34. Chambaud I, Wóblewski H, Blanchard A. Interactions between mycoplasma lipoproteins and the host immune system. Trends Microbiol 1999, 7(12):493-499.

35. Broadway MM, Rogers EA, Chang C, Huang J-H, Dwivedi P, Yildirim S, et al. Pilus gene pool variation and the virulence of Corynebacterium diphtheriae clinical isolates during infection of a nematode. J Bacteriol 2013, 195(16):3774-3783.

36. Baker JL, Sudarsan N, Weinberg Z, Roth A, Stockbridge RB, Breaker RR: Widespread genetic switches and toxicity resistance proteins for fluoride. Science 2012, 335(6065):233-235.

37. Mukamolova GV, Turapov OA, Young DJ, Kapelyants AS, Kell DB, Young M: A family of autocrine growth factors in Mycobacterium tuberculosis. Mol Microbiol 2002, 46(3):623-635.

38. Marcyjaniak M, Odintsov SG, Sabala I, Bochtler M. Peptidoglycan amidase MepA is a LAS metalloproteinase. J Biol Chem 2004, 279(42):43982-43989.

39. Tomioka S, Matsushashi M. Purification of penicillin-insensitive DD-endopeptidase, a new cell wall peptidoglycan-hydrolyzing enzyme in Escherichia coli, and its inhibition by deoxyribonucleic. Biochem Biophys Res 1978, 84(4):978-984.

40. Guimarães LC, Silva NF, Miyoshi A, Schneider MPC, Silva A, Azevedo V, et al. Structure modeling of a metalloendopeptidase from Corynebacterium pseudotuberculosis. Comput Biol Med 2012, 42(5):538-541.

41. Sutcliffe IC, Harrington DJ. Lipoproteins of Mycobacterium tuberculosis: an abundant and functionally diverse class of cell envelope components. FEMS Microbiol Rev 2004, 28(5):645-659.

42. Schröder J, Maus I, Meyer K, Wördemann S, Blom J, Jarnicke S, et al. Complete genome sequence, lifestyle, and multi-drug resistance of the human pathogen Corynebacterium resistens DSM 45100 isolated from blood samples of a leukemia patient. BMC Genomics 2012, 13:141.

43. Gaspar AH, Ton-That H. Assembly of Distinct Pilus Structures on the Surface of Corynebacterium diphtheriae Assembly of Distinct Pilus Structures on the Surface of Corynebacterium diphtheriae. 2006, 188(4):1526-1533.

44. Karp PD, Paley SM, Krummenacker M, Latendresse M, Dale J, Lee T, et al. Pathway Tools version 13.0: integrated software for pathway/genome informatics and systems biology. Briefings Bioinforma 2010, 11(1):40-79.

doi:10.1186/1471-2164-16-S5-S7
Cite this article as: Guimarães et al.: Genome informatics and vaccine targets in Corynebacterium urealyticum using two whole genomes, comparative genomics, and reverse vaccinology. BMC Genomics 2015 16(Suppl 5):S7.