Research paper

Complete genome analysis of *Glutamicibacter creatinolyticus* from mare abscess and comparative genomics provide insight of diversity and adaptation for *Glutamicibacter*

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ABSTRACT

Bacteria of the genus *Glutamicibacter* are considered ubiquitous because they can be found in soil, water and air. They have already been isolated from different habitats, including different types of soil, clinical samples, cheese and plants. *Glutamicibacter creatinolyticus* is a Gram-positive bacterium important to various biotechnological processes, however, as a pathogen it is associated to urinary tract infections and bacteremia. Recently, *Glutamicibacter creatinolyticus* LGCM 259 was isolated from a mare, which displayed several diffuse subcutaneous nodules with heavy vascularization. In this study, sequencing, genomic analysis of *G. creatinolyticus* LGCM 259 and comparative analyses were performed among 4 representatives of different members of genus from different habitats, available in the NCBI database. The LGCM 259 strain's genome carries important factors of bacterial virulence that are essential in cell viability, virulence, and pathogenicity. Genomic islands were predicted for 4 members of genus *Glutamicibacter*, showing a high number of GEIs, which may reflect a high interspecific diversity and a possible adaptive mechanism responsible for the survival of each species in its specific niche. Furthermore, *G. creatinolyticus* LGCM 259 shares syntenic regions, albeit with a considerable loss of genes, in relation to the other species. In addition, *G. creatinolyticus* LGCM 259 presents resistance genes to 6 different classes of antibiotics and heavy metals, such as: copper, arsenic, chromium and cobalt-zinc-cadmium. Comparative genomics analyses could contribute to the identification of mobile genetic elements particular to the species *G. creatinolyticus* compared to other members of genus. The presence of specific regions in *G. creatinolyticus* could be indicative of their roles in host adaptation, virulence, and the characterization of astrain that affects animals.

1. Introduction

Glutamicibacter creatinolyticus, previously described as *Arthrobacter creatinolyticus*, belonged to the genus *Arthrobacter* of the family *Micrococcaceae*. The new reclassification proposal occurred in 2016, where the genera of the family *Micrococcaceae* were renamed as *Glutamicibacter*, *Paeniglutamicibacter*, *Pseudoglutamicibacter*, *Paenarthrobacter* and *Pseudarthrobacter* (Busse, 2016).

The environmental prevalence of *Glutamicibacter s*p. strains can be considered to be due to their nutritional versatility and ability to respond to environmental stresses (Yao et al., 2015). It is unsurprising that strains of the genus *Glutamicibacter* are phenotypically heterogeneous and have been isolated from distinct sources, such as soil (Shen et al., 2013), clinical specimens (Hou et al., 1998; Yamamoto et al., 2017), cheese (Monnet et al., 2010), and plants (Feng et al., 2017). Bacteria of the genus *Glutamicibacter* are thought to play a significant role in many ecosystems and to affect human welfare (Yamamoto et al., 2017). The currently identified *Glutamicibacter* species are *Glutamicibacter nicotianae*, *Glutamicibacter arilaitensis*, *Glutamicibacter halophytocola*, *Glutamicibacter soli*, *Glutamicibacter mysorens* and Glutamicibacter creatinolyticus (Information acquired from the NCBI Database).

The species *G. creatinolyticus* is thought to be associated to biotechnological processes and has been reported in a limited number of clinical cases related to infection processes such as bacteremia and urinary tract infections (Hou et al., 1998, Yamamoto et al., 2017). The general morphological characteristics of *Glutamicibacter creatinolyticus* have been described as follows: circular colonies, smooth and pigmented yellow when grown on brain heart infusion agar (BHI), the bacteria are endospore negative, non-motile, creatinine hydrolysis positive, shaped like irregular rods, aerobic, catalase-positive, and have a genomic G + C content of 66–67% (Hou et al., 1998). The peptidoglycan composition and quinone system are in accordance with the genus' description, but its polar lipid and fatty acid profiles are unknown (Busse, 2016). *G. creatinolyticus* as a bioremediation treatment, works efficiently in the decontamination of arsenic contaminated water, through the interaction between plants and bacteria (Prum et al., 2018). This species has shown to be a potent producer of extracellular urease for the determination of heavy metal ions (Ramesh et al., 2014) and been deployed as a potentiometric biosensor for the determination of urea content in milk, using immobilized urease (Ramesh et al., 2015).

Likewise, *G. creatinolyticus* strains have been isolated from human urine with unusually low creatinine concentrations, due to their ability to hydrolyze creatinine (Hou et al., 1998). Strains of this species were also isolated from an elderly diabetic man with acute cholangitis, to whom this species caused bacteremia (Yamamoto et al., 2017). *G. creatinolyticus* strain LGCM 259 was recently isolated from diffuse subcutaneous nodules and masses from a mare in Italy (RIFICI et al., 2019, article accepted for publication).

Due to the inefficiency of classical biochemical methods in the taxonomic classification of this species, better prediction strategies were required, such as 16S rRNA and MALDI Biotyper sequence analyses (Funke et al., 1996). Currently, three species have complete genomes and 9 have incomplete genomes without a definite species deposited in the National Center for Biotechnology Information (NCBI) database.

In this study, we present the first complete genome of the species *G. creatinolyticus* LGCM 259. The comprehensive analyses of this genome sequence provide better ways of understanding the molecular and genetic basis of this species. Comparative genomic analyses between species could allow for the identification of mobile genetic elements (e.g. pathogenicity islands and bacteriophage sequences) as genetic characteristics determinant of the completely different habitats described for each species of the genus. In addition, phylogenomic analyses allow for the verification of the taxonomic reclassification of all completely sequenced strains of members of genera *Arthrobacter* and *Glutamicibacter*.

2. Material and methods

2.1. Bacterial culture and DNA extraction from G. creatinolyticus

G. creatinolyticus strain LGCM 259 was previously isolated from the abscess of a 12-year-old mare in Italy (RIFICI et al., 2019, article accepted for publication). In order to extract the genomic DNA, this strain was first cultivated on BHI (Brain Heart Infusion) agar and, next, on 30 mL of BHI broth, at 37 °C, overnight. The culture was centrifuged for bacterial pellet formation and the supernatant was discarded. The pellet was suspended in 600 μL of solution (Tris-HCI pH 7.0, 0.5 M EDTA pH 8.0, NaCl 5 M, and distilled H2O enough to obtain 50 mL) and transferred to a 2 mL tube containing glass beads (VK01) (Bertin Technologies) to subjecting bacteria to mechanical lysis. Two homogenization cycles of 15sec each, at 6500 rpm, were performed using Precellys 24 (Bertin Technologies). Subsequently, 1 mL of phenol: chloroform: isoamyl alcohol (25:24:1) solution was added to the tube and the mixture was homogenized and centrifuged at 13,000 rpm, for 7 min. Next, the upper aqueous phase of the mixture was transferred to a new tube, and a second round of phenol: chloroform: isoamyl alcohol purification was performed. Next, the upper aqueous phase was recovered and mixed with 1 mL of chloroform. Following centrifugation at 13,000 rpm, for 7 min, the upper aqueous phase was transferred to a new tube. 1 mL of ethyl alcohol, 40 μL of 3 M NaAc, and 4 μL of 20 mg/ mL glycogen were added. Following gentle inversion, the mixture was placed at −20 °C, overnight, for DNA precipitation. Following centrifugation at 13,000 rpm, for 15 min, the supernatant was discarded. 1 mL of 70% ethyl alcohol was added to rehydrate the DNA pellet. A second round of 70% ethyl alcohol wash was performed using centrifugation at 13,000 rpm, for 15 min. The DNA pellet was placed at 60 °C to dry. Finally, the DNA precipitate was suspended in 50 μL of sterilized ultra-pure water. DNA quantity and quality assessments were conducted using NanoDropTM 2000 (Thermo ScientificTM), Qubit Fluorometer (Thermo ScientificTM) and 1% agarose gel electrophoresis (Pacheco et al., 2007).

2.2. Genome sequencing, assembly and annotation

Chromosomal sequencing of *G. creatinolyticus* strain was performed using Hiseq technology (Illumina, San Diego, CA, USA), using paired-end libraries (2 × 150 bp). The ab initio assembly was performed using the software Spades, version 3.9 (Bankevich et al., 2012). For the choice of the best references, the contigs generated for the strain were submitted to the Basic Local Alignment Search Tool for nucleotides (BLASTn) (Altschul et al., 1990). The assembled contigs were oriented to generate a scaffold using Medusa v.1.3 (Bosi et al., 2015), and the strains *Arthrobacter* sp. YC-RL1 and *Arthrobacter/Glutamicibacter arilaitensis* Re117 were used as reference. Gaps resulting from the assemblies were manually filled using the CLC Genomics Workbench software, for visualization, version 7.0 (Qiagen, USA), where the reads were mapped against a reference genome to generate a consensus sequence, which was then used to close the gaps. The genome of the *G. creatinolyticus* LGCM 259 was automatically annotated using PROKKA v3 (Seemann, 2014) and deposited in National Center for Biotechnology Information database (NCBI) (https://www.ncbi.nlm.nih. gov).

2.3. Complete genomes of the Arthrobacter genus and its new reclassification available for comparative genomics analysis

The sequences of 26 genomes were downloaded from NCBI (Table 1). The software Prokka was used to homogenize the genome annotation to perform the comparative analyses (Seemann, 2014).

2.4. Phylogenomic analyses

The Taxonomical Revision, a proposal to reclassify the species of the genus *Arthrobacter*, has divided it into 5 different genera (Busse, 2016). Due to this new classification, we performed a phylogenomic analysis, using all complete genomes from the *Arthrobacter* genus, as well as the new genera *Glutamicibacter*, *Pseudoarthrobacter*, and *Paenarthrobacter* that were available in the NCBI database (Table 1). The other two genera (*Paeniglutamicibacter* and *Pseudoglutamacibacter*) have not been available complete genomes. For that purpose, a phylogenomic tree was generated using the Phylogenomic Tree Tool in Pathosystems Resource Integration Center (PATRIC) (http://www.patricbrc.org), version 3.5.17 (Wattam et al., 2014). The Maximum Likelihood method was used, with the Automated Progressive Refinement option selected. *Micrococcus luteus* NCTC 2665 was used as an outgroup. The software MEGA X: Molecular Evolutionary Genetics Analysis was used to construct the Maximum-likelihood tree using the 16S gene sequence (Kumar et al., 2018).

2.5. Average nucleotide identity (ANI) and tetra nucleotide

Average nucleotide identity (ANI) and tetra nucleotide frequencies were estimated by JSpecies Web Server (available at http://imedea.uib-csic.es/jspecies/about.html) (Richter et al., 2016) based on BLAST (Altschul et al., 1990), selecting the ANIb and Tetra nucleotide options. In a given genome pair, the ANI performs pairwise comparisons of 1020 bp fragments between a query and a reference genome (Tindall et al., 2010). The tetra nucleotide frequencies perform signatures between paired genome comparisons (Tindall et al., 2010). Typically, the threshold frontier to consider two organisms to belong to the same species could be set at > 95% identity for ANI and > 99% for Tetra (Busse et al., 2010; Richter et al., 2016).

2.6. Identification of genes encoding virulence factors

The presence of virulence genes in the genome was identified using BLASTp (Altschul et al., 1990) against the Virulence Factor Database (VFDB) (Chen et al., 2004). The parameters considered were an E-value of 1e-5, a minimum identity percentage of 50% and minimum coverage of 70%, between the query and subject sequences (Lindahl and Elofsson, 2000; Yang and Honig, 2000). The functional annotations were obtained from the categories provided by the Virulence Factor Database (Chen et al., 2004).

2.7. Genome plasticity analysis of G. creatinolyticus compared to other Glutamicibacter

Genomic island (GEIs) predictions were performed using Genomic Island prediction Software (GIPSy), version 1.1.2 (Soares et al., 2016), using the genome of *G. arilaitensis* strain Re117 as a reference. Gipsy classifies GEIs into four different categories: (i) pathogenicity islands (PAIs), which carry virulence factor genes (Dobrindt and Hacker, 2001); (ii) metabolic islands (MIs), which harbor genes associated to the biosynthesis of (secondary) metabolites (Tumapa et al., 2008); (iii) resistance islands (RIs), containing genes that code for resistance, typically against antibiotics (Krizova and Nemec, 2010); and symbiotic islands (SIs), facilitating symbiotic associations of the host with other micro- and microorganisms (Barcellos et al., 2007). In addition, we evaluated the presence of genomic islands for each species of the genus *Glutamicibacter*. To define homologous GEIs, we considered an identity higher than 60%. Finally, BLAST Ring Image Generator (BRIG), version 0.95 (Alikhan et al., 2011), was used to map the GEIs. To identify conserved genomic regions among species of genus *Glutamicibacter*, including DNA rearrangements and inversions, a synteny analysis was conducted using Mauve (Darling et al., 2004), version 2.4.0, with the most accurate option for alignment selected, Progressive Mauve. For

this analysis, sequences from the chromosome of strains *A. sp.* YCRL1 isolated from petroleum-contaminated soil, *G. halophytocola* KLBM5180, isolated from the roots of the plant *Limonium sinense* (Feng et al., 2017), *G. nicotinae* OTC16 isolated from active sludge around pharmaceutical company (Wang et al., 2015), and *G. arilaitensis* RE117isolated from the surface of cheeses (Monnet et al., 2010).

3. Results

### 3.1. G. creatinolyticus general genomic features

The *G. creatinolyticus* LGCM 259 chromosome sequence has been deposited in the NCBI database under accession number CP034412. The strain was sequenced and assembled in a circular chromosome, which exhibits a length of 3,3 Mb, with a G + C content of 66.4%, and a total of 2882 CDSs, 4 clusters of rRNAS (5S, 16S, and 23S), and 61 tRNA genes, respectively. To further analyze the genome of *G. creatinolyticus* LGCM 259, functional characterization of genome sequences was realized with Kyoto Encyclopedia of Genes and Genomes (KEGG) using BLASTKOALA (Kanehisa et al., 2016). Gene clustering analysis (Supplementary table 1), revealed the presence of a total of 1487 KEGG-associating genes; the top categories belonged to carbohydrate metabolism (174 genes; 11,58%); protein families: genetic information processing (173 genes; 11.51%), genetic information processing (161 genes; 10.71%); signaling and cellular processes (155 genes; 10.31%); amino acid metabolism (133 genes; 8.85%); environmental information processing (104 genes; 6.92%), unclassified: metabolism (82 genes; 5.46%), metabolism of cofactors and vitamins (81 genes; 5.39%), and other categories (440 genes; 29.27%), respectively (Fig. 1).

3.2. Phylogenomic analysis with G. creatinolyticus

Phylogenomic analyses were performed between the completed sequence of strain LGCM 259 and twenty-six (Table 1) other whole bacterial genomes previously described. The phylogenetic tree based on core genes was constructed using the Maximum Likelihood method (Fig. 2). Additionally, a phylogenetic analysis using 16S rRNA genes was performed (Supplementary Fig. 1). In both comparisons, the

Table 1
Genomes downloaded from the NCBI used in this work.

N°	Strain Name	Accession	Size (bp)
1	*Arthrobacter sp.* Rue61a	PRJNA78011	4,736,495
2	*Arthrobacter* sp. FB24	PRJNA12640	4,698,945
3	*Arthrobacter* sp. PAMC 25486	PRJNA244892	4,593,579
4	*Arthrobacter* sp. ERGS1:01	PRJNA293260	4,030,317
5	*Arthrobacter* sp. YC-RL1	PRJNA302833	3,846,272
6	*Arthrobacter* sp. ATCC 21022	PRJNA306041	4,434,904
7	*Arthrobacter* sp. U41	PRJNA320863	4,386,369
8	*Arthrobacter* sp. ZXY-2	PRJNA341911	4,495,402
9	*Arthrobacter* sp. QXT-31	PRJNA361372	5,041,568
10	*Arthrobacter* sp. YN	PRJNA393603	5,063,552
11	*Arthrobacter* sp. Hiyo4	PRJDB3373	3,779,248
12	*Arthrobacter* sp. PGP41	PRJNA431708	4,270,237
13	*Arthrobacter* sp. DCT-5	PRJNA473399	4,359,122
14	*Arthrobacter* sp. Hiyo8	PRJDB3373	4,698,617
15	*Glutamicibacter arilaitensis*Re117	PRJNA224116	3,859,257
16	*Glutamicibacter halophytocola* KLBMP 5180	PRJNA289022	3,911,798
17	*Glutamicibacter nicotianae* OTC-16	PRJNA490584	3,643,989
18	*Arthrobacter crystallopoietes* DSM 20117	PRJNA357926	5,032,705
19	*Arthrobacter alpinus*R3.8	PRJNA295631	4,046,453
20	*Pseudarthrobacter phenanthrenivorans* Sphe3	PRJNA295631	4,046,453
21	*Paenarthrobacter aurescens* TC1	PRJNA12512	4,597,686
22	*Arthrobacter radiotolerans* RSPS-4	PRJNA241417	3,267,233
23	*Pseudarthrobacter equi*	PRJEB16401	4,459,178
24	*Pseudarthrobacter sulfonivorans* Ar51	PRJNA305788	5,043,757
25	*Pseudarthrobacter chlorophenolicus* A6	PRJNA20011	4,980,870
26	*Micrococcus luteus* NCTC 2665	PRJNA20655	2,501,097
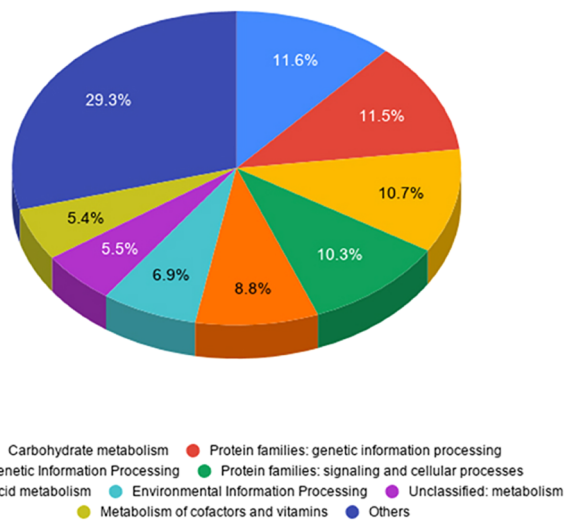
Fig. 1. Functional Annotation of *G. creatinolyticus* LGCM 259 genes. This figure was prepared by analysis of the whole genome and relative abundance and distribution of KEGG categories plotted using Microsoft Excel.

species *Arthrobacter radiotolerans* had the most divergent genome in relation to the closest available complete sequences of the same family found in the database, once this species has already been reclassified as *Rubrobacter radiotolerans* (Egas et al., 2015). In both approaches, it is possible to see that the *G. creatinolyticus* LGCM 259 forms a clade with its respective genus (Fig. 2). The genome of *Arthrobacter* sp. YCRL1, was grouped with the *Glutamicibacter* genus, suggesting that this strain may be part of the genus. However, more studies need to be performed, with genomes representing every genus, in order to reclassify all these

genomes in accordance to the new classification suggested by Busse (2016). The resulting Newick tree file was visualized using iTOL v4.261 (Letunic and Bork, 2016). Thus, all comparative analyses were performed using the complete genomes representatives of the members of the genus *Glutamicibacter* and the genome of *A. sp* YCRL1, because it is so close to the genus.

3.3. Average nucleotide identity (ANI) and tetra nucleotide

In order to better understand how strain LGCM 259 is genetically correlated with the genomes of other genera, formerly known as *Arthrobacter* and the new genera proposed by Busse (2016), mean nucleotide identity predictions were conducted, using all completed genomes, encompassing the entire *Arthrobacter* genus and its new classification, that include the 3 different species of *Glutamicibacter* genus downloaded from NCBI (Busse, 2016). All genomes displayed a low degree of similarity with the reference strain, *G. creatinolyticus* LGCM 259 (ANI < 80% and tetra nucleotide < 96%), thus, we can hypothesize that none of genomes belonged to the same species (Supplementary figures 2 and 3).

3.4. Virulence factors (VFs) predicted in G. creatinolyticus may have a role in infection

We identified 10 virulence factors (VFs) in the genome, detailed in (Supplementary Table 2). We identified the gene, isocitrate lyase activity (ICL) (locus tag: LGCM259_0286), that is related the metabolism of fatty acids and necessary for the assimilation of acetates in *Rhodococcus equi* (Wall et al., 2005). Isocitrate lyase activity (ICL) were previously studied in other organisms including *M. tuberculosis* and have been associated with increased virulence (Muñoz-Elías and McKinney, 2005).
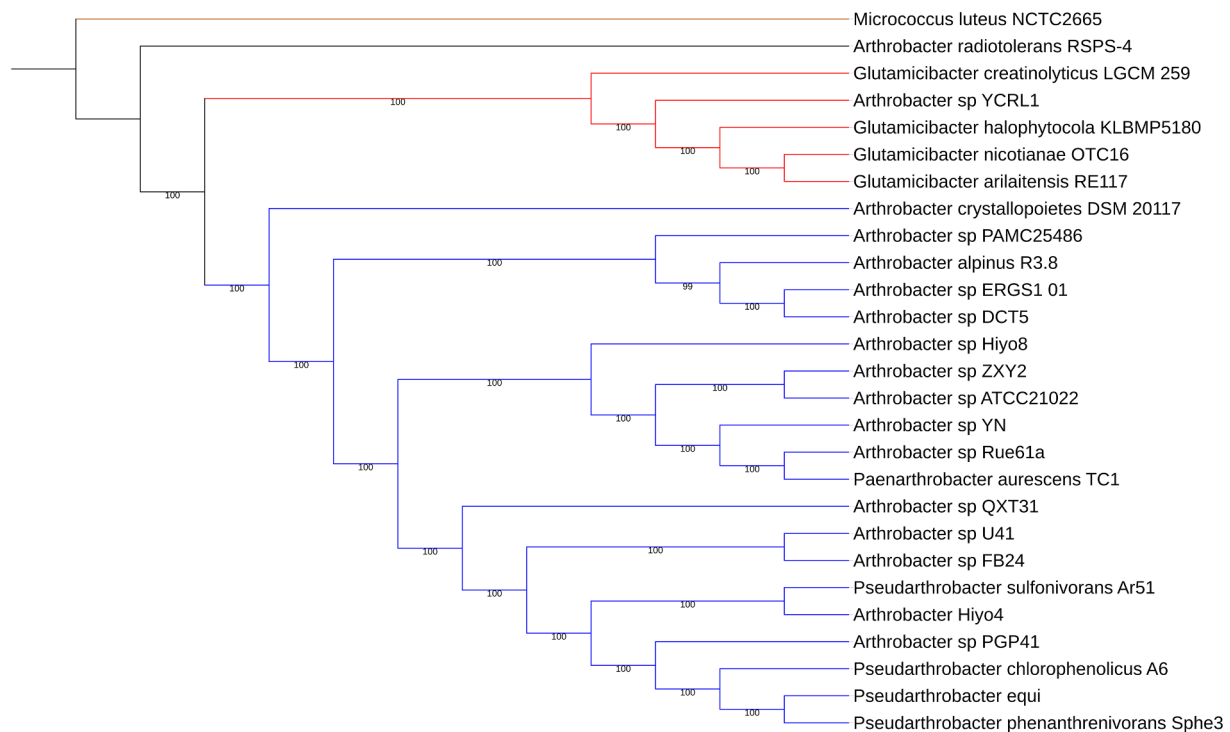
Fig. 2. Phylogenomic analysis based in the Maximum Likelihood method. The *Glutamicibacter* genus is highlighted in red, along with the genome of *G. creatinolyticus* LGCM 259, and *A. sp.* YCRL1. *Arthrobacter radiotolerans* is the most divergent strain in the tree. The genome de *Micrococcus luteus* NCTC 2665, members of *Micrococcaceae* family was used as an outgroup. The numbers represent the bootstrap values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Bacterial ABC transporters involved in cell viability, virulence, and pathogenicity were also identified. We identified a cluster of genes coding for enzymes involved in ABC transportation, which includes groEL (locus tag: LGCM259_0465), *groES* (locus tag: LGCM259_ 0460) and *fepG* (locus tag: LGCM2_0733). The *groEL* gene prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions. To function properly, *groEL* requires the lid-like cochaperonin protein complex GroES (*groES* gene) (Maguire et al., 2002). Already *fepG* is part of the genes involved in the iron transport, important elements for growth and bacterial metabolism (Bunet et al., 2006). We also identified the virulence factor involved in two-component signal transduction systems (TCSs) (locus tag: LGCM259_0734), allows bacteria to sense, respond, and adapt to changes in their environment or in their intracellular state (Othmer et al., 2013). TCSs usually consist of a membrane-bound sensor histidine protein kinase (HPK) that perceives environmental stimuli, and a response regulator (RR) that affects gene expression (Yamamoto et al., 2005). This virulence factor is involved in metal ions fulfill, a plethora of essential roles within bacterial pathogens. Aside of being indispensable for the structure and for the function of proteins, they also fulfill roles in signaling and virulence regulation (Begg, 2019). We identified the *ideR* (locus tag: LGCM259_0465) gene acts as a siderophore biosynthesis repressor and as an iron storage positive modulator (Gold et al., 2001). A protein involved in kinase activity, the GTP pyrophosphokinase RelA (locus tag: LGCM259_1510), a stringent response mediator that coordinates a variety of cellular activities in response to changes in nutritional abundance, also was identified (Edwards et al., 2011). In this analysis we find the UDP-galactose 4-epimerase (GalE) (locus tag: LGCM259_1204) which is also called UDP-glucose 4-epimerase, is an enzyme responsible for interconversion of UDP-galactose and UDP-glucose (Li et al., 2014). GalE is also an important virulence factor in a few bacterial pathogens (Li et al., 2014). Other VFs involved in pentose-phosphate shunt and Peptidyl-prolyl cis-

trans isomerase or PPIase (Unal and Steinert, 2014) (cypB gene locus tag: LGCM259_0015) were found. PPIases accelerate the folding of proteins and have also been identified as virulence-associated proteins. The extent of their contribution to virulence is highly variable and dependent on the pleiotropic roles of a single PPIase in the respective pathogen (Unal and Steinert, 2014).

3.5. Genome reduction and unique genomic island profile of G. creatinolyticus compared to other Glutamicibacter

Comparative analysis using MAUVE evidenced an overall structural conservation and collinearity among the chromosomes of the different genomes (Fig. 3). The multiple alignments showed the existence of locally collinear blocks (LCBs). However, it was observed that specific regions of each genome were present in the LCBs (white segments), which may harbor specific adaptations of each bacterium and infer adaptation by species (Darling et al., 2004). These regions probably represent DNA acquired during Horizontal Gene Transfer (HGT) events and may provide a greater metabolic versatility to LGCM 259 strain. Regions that are inverted in relation to the strain LGCM 259 are shown as dislocated below the central genome axis. Regarding the size of the chromosome, the LGCM 259 strain chromosome presented to be smaller (3.3 Mpb) than the four other species used for this work (3.8, 3.8, 3.9, 3.6 Mbp para *A. sp.*YCRL1, *G. arilaitensis* RE117, *G. halophytocola* KLBM5180, and *G. nicotinae* OTC16, respectively). The genome of *G. halophytocola* KLBM5180 showed the largest chromosome size. This is probably due to the accumulation of genes related to their adaptation to a different ecological niche, since this strain is a bacterium capable of producing a wide range of secondary biological metabolites and their beneficial effects on host plants (Feng et al., 2017). The GC content of strain LGCM 259 strain is 66.4%, higher than the other genomes (64.26, 59.29, 60.91, 62.92% *A. sp.* YCRL1, *G. arilaitensis* RE117, *G. halophytocola* KLBM5180, and *G. nicotinae* OTC16, respectively).
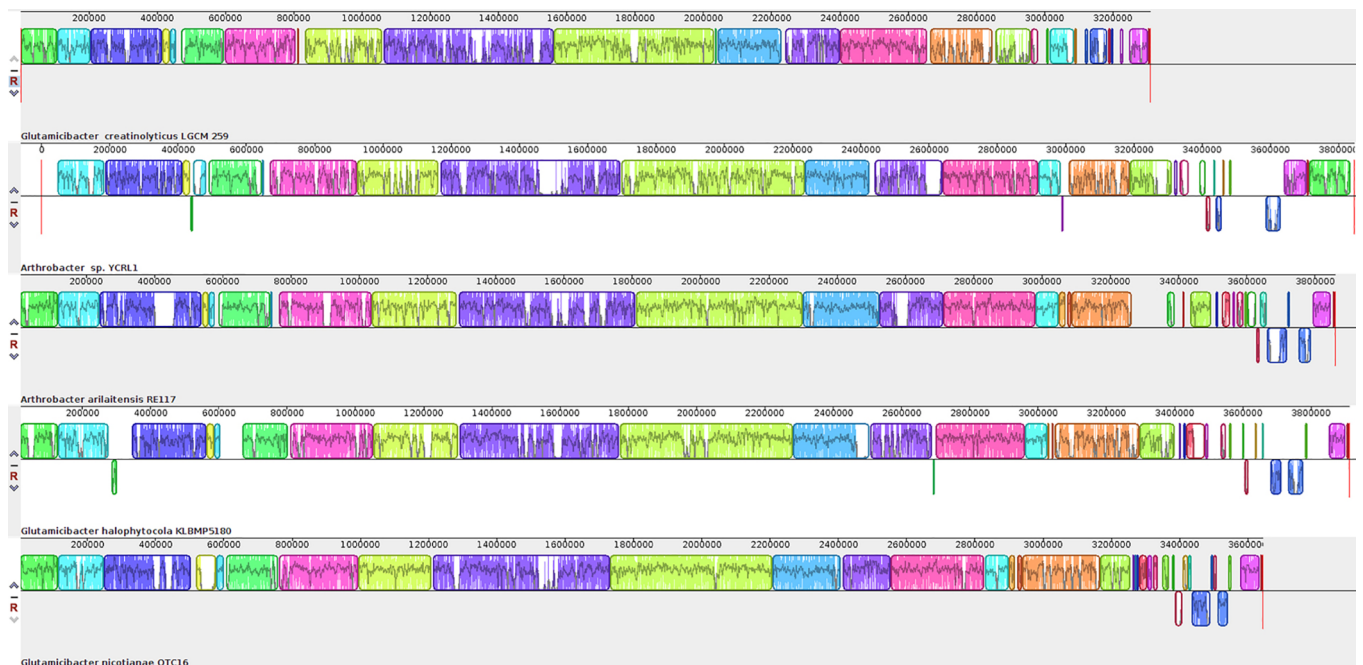
Fig. 3. Whole genome comparative alignments between *G. creatinolyticus* LGCM 259 with *A. sp.* YCRL1, *G. arilaitensis* RE117, *G. halophytocola* KLBM5180, and *G. nicotinae* OTC16. Each analyzed genome is displayed horizontally, with each conserved segment represented by colored blocks, connected between each genome, and sequence coordinates are shown in scale. The aligned region is in the forwards orientation in relation to the first genome sequence if a block is above the central line, and blocks below the central line indicate regions aligned in the complementary inverted orientation. Regions not belonging to any blocks are regions that do not show significant homology between the analyzed genomes. Regions in white are unique to each species.

Another interesting trait of the strain LGCM 259 is that the genome presented several genomic islands. The number of predicted GEIs was 23 (Fig. 4). Of these 23 GEIs, 15 islands were pathogenic, 4 resistance, 2 metabolic and 2 symbiotic islands. Curiously, the GEIs displayed a different GC content from the rest of the genome, as well as factor codifying products typically involved in genetic mobility, such as integrases, recombinase and transposases, this suggests that some GEIs were likely recruited through Horizontal Gene Transfer (HGT), to facilitate the bacterium's survival in diverse niches (Bellanger et al., 2014). These resources are commonly incorporated in GEI prediction markers (Bellanger et al., 2014). Additionally, the GEIs displayed genes implicated Arsenical-resistance protein, Vancomycin resistance protein VanJ). Bacterial Horizontal Gene Transfer can be greatly advantageous to the bacterium. This can contribute to the generating novel metabolic functions, genomic plasticity and adaptive value. Therefore, it plays a fundamental role in bacterial evolution (Gal-Mor and Finlay, 2006).

The genomes of 4 species of the genus *Glutamicibacter* and a genome of the *Arthrobacter* genus were analyzed for the prediction of GEIs. This analysis showed a considerable variation in the number of genomic islands (Supplementary Table 3). GEIs were predicted for each genome, with each *Glutamicibacter* genome containing, in average, 25 GEIs. Each species was represented by a strain isolated from a specific niche. Through GEI prediction, we hoped to identify genes in the islands functionally related to the bacterium's specialization to their specific niche.

The comparative analysis between the GEIs of *G. creatinolyticus* LGCM 259 identified 10 specific GEIs for this species (Supplementary Table 4) (Fig. 2). Of these 10 exclusive genomic islands, 5 are PAIs. For PAIs 4 and 5, products were reported (Table 2), of which most are involved in sugar transportation, transcriptional regulators, and the products microssistin degradation and copper homeostasis. In addition, the island MI2 was identified, displaying genes involved in the regulation and structure of the bacterium's lipopolysaccharide cover. These genes may be important to the species' classification and identification. Furthermore, 3 RIs, 1 MI and 1 SI were reported, of which SI2 displayed products related to transcriptional regulation, cobalt metabolism and amino acid metabolism (Table 2).

In the species *G. arilaitensis*RE117identified 10 specific GEIs for this species (Supplementary Table 5; Supplementary Fig. 4). We report 5 unique pathogenic islands, of which PAI3 and PAI12 displayed some proteins, mainly involved in oxidative stress, metal transport, transcriptional regulation, amino acid metabolism, and a toxin-antitoxin system virulence factor (Table 3), (Zhang et al., 2017; Monnet et al., 2010). Genes involved in the environmental adaptation to cheese were not reported in genomic island content, which may be due to their transmission being chromosomal, not having been acquired from HGT events (Bonham et al., 2017).

In the species *A. sp.* YRLC1, we report 10 specific GEIs (Supplementary Table 6: Supplementary figure 5), them being: 1 pathogenic, 3 of resistance, and 4 metabolically. Of these, MI3 displays proteins involved in electron transportation, which are important in soil with high mineral content. (Table 3) (Zhang et al., 2017). MI6 displayed some products involved in sugar transportation and metabolism, which are involved in nutrient rich soil (Table 3). 2 SIs were also found,

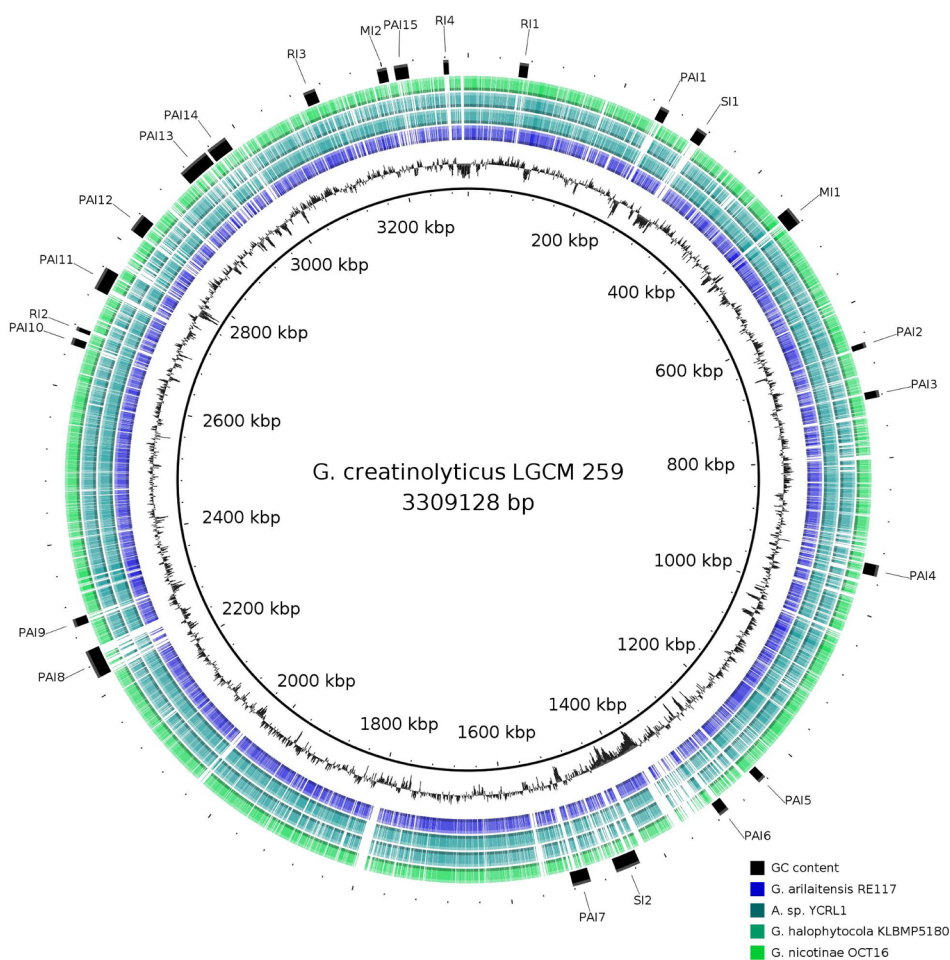**Fig. 4.** Circular genome map representing all genomic islands found in the *G. creatinolyticus* LGCM 259 genome. Representation, from the inner to the outer circle, is: *G. creatinolyticus* LGCM 259, GC Content, *G. arilaitensis*Re117, *A. sp.* YCRL1, *G. hlophytocola*KLBM5180 and *G. nicotinae*OTC16. Pathogenicity islands (PAIs); Metabolic islands (MIs), Resistance islands (RIs), and symbiotic islands (SIs).

Table 2

Description of the products of some exclusive islands of *G. creatinolyticus* LGCM 259.

Strain Name	Exclusive island	Products
G. creatinolyticus LGCM 259	PAI4	Cadmium-transporting ATPase, Copper chaperone CopZ, cAMP receptor protein, HAD family hydrolase, Hypothetical protein, Restriction endonuclease, Microcystin degradation protein MlrC
G. creatinolyticus LGCM 259	PAI5	Fatty acid desaturase, hypothetical protein, Cold shock protein, IS1380 family transposase, LacI family transcriptional regulator, Alpha-amylase, ABC transporter substrate-binding protein, Maltose ABC transporter permease, Sugar ABC transporter permease, 3,4-dihydroxyphenylacetate 2,3-dioxygenase
G. creatinolyticus LGCM 259	MI2	16S ribosomal RNA, 23S ribosomal RNA, 5S ribosomal RNA, Dehydrogenase, Formate dehydrogenase, nitrate-inducible, major subunit precursor, Formate dehydrogenase, nitrate-inducible, iron-sulfur subunit, Polysulfide reductase NrfD, Selenide, water dikinase, Transposase, tRNA-Sec, L-seryl-tRNA(Sec) selenium transferase, Selenocysteine-specific elongation factor, Trehalose 6-phosphate phosphatase
G. creatinolyticus LGCM 259	SI2	XRE family transcriptional regulator, Alanine racemase, Aspartate aminotransferase family protein, Recombinase, Amino acid permease, cobT Aerobic cobaltochelatase subunit CobT, IclR family transcriptional regulator, Sulfoacetaldehyde acetyltransferase

Table 3

Description of the products of some exclusive islands of *G. arilaitensis* RE117 and *A. sp.* YRLC1.

Strain Name	Exclusive island	Products
G. arilaitensis RE117	PAI3	recombinase family protein, hypothetical protein, single-stranded DNA-binding protein, type VI secretion protein, hypothetical protein, ATP-binding protein, hypothetical protein, ABC transporter ATP-binding protein, Zn-dependent oxidoreductase, membrane protein, class I SAM-dependent methyltransferase, M23 family metallopeptidase, chromosome partitioning protein ParB, DUF2637 domain-containing protein, DNA primase, ImmA/IrrE family metallo-endopeptidase, AraC family transcriptional regulator, 6 ABC transporter ATP-binding protein, iron ABC transporter permease, siderophore-interacting protein, helix-turn-helix domain-containing protein, DNA cytosine methyltransferase, conjugal transfer protein, DNA-processing protein DprA, IS3 family transposase, integrase
G. arilaitensis RE117	PAI12	IclR family transcriptional regulator, dihydroorotate dehydrogenase, MFS transporter, Zn-dependent hydrolase, dihydroorotate oxidase electron transfer subunit, dihydroorotase, aspartate ammonia-lyase, cyclohexadienyl dehydratase, SDR family oxidoreductase, MFS transporter, transketolase, GntR family transcriptional regulator, transcriptional regulator, type II toxin-antitoxin system HipA family toxin, alkene reductase, SDR family oxidoreductase.
A. sp. YRLC1	MI3	cyclic amidohydrolase, ribonuclease BN, hypothetical protein, monooxygenase, glutaminase, aldehyde dehydrogenase, hydrolase, ferredoxin, dehydrogenase, phosphoenolpyruvate synthase, cytochrome, IclR family transcriptional regulator, metal-dependent phosphohydrolase, monooxygenase, formate–tetrahydrofolate ligase
A. sp. YRLC1	MI6	glycosyltransferase, hypothetical protein, acetyltransferase, CAAX protease, alkaline phosphatase, sugar ABC transporter permease, sugar ABC transporter substrate-binding protein, fructose-bisphosphate aldolase, hypothetical protein, sugar isomerase
A. sp. YRLC1	SI1	hypothetical protein, iron-binding protein, ABC transporter, phosphonate ABC transporter permease, phosphonate ABC transporter, phosphonate ABC transporter substrate-binding protein, oxidoreductase, GntR family transcriptional regulator, HAD family hydrolase, transcriptional regulator

Table 4

Description of the products of some exclusive islands of *G. nicotinae* OCT16 and *G. halophytocola* KLMB5180.

Species	Exclusive island	Products
G. nicotinae OCT16	MI1	amino acid permease, hypothetical protein, TRAP transporter large permease subunit, NAD(P)-dependent oxidoreductase, methyltransferase, IclR family transcriptional regulator, tripartite tricarboxylate transporter TctB family protein, tripartite tricarboxylate transporter permease, tripartite tricarboxylate transporter substrate binding protein, lycoside hydrolase family 3 protein, TRAP transporter large permease, TRAP transporter small permease, LacI family transcriptional regulator, glucuronate isomerase, mannitol dehydrogenase family protein, D-galactonate dehydratase family protein
G. nicotinae OCT16	SI3	glycerate kinase, MFS transporter, SDR family oxidoreductase, M20 family peptidase, PLP-dependent aminotransferase family protein, M24 family metallopeptidase, LysR family transcriptional regulator, aspartate aminotransferase family protein, NAD-dependent succinate-semialdehyde dehydrogenase, ald alanine dehydrogenase, ulP family inorganic anion transporter, GGDEF domain-containing protein, EAL domain-containing protein, glycosyltransferase family 2 protein
G. halophytocola KLMB5180	MI1	LacI family transcriptional regulator, MFS transporter, mandelate racemase/muconate lactonizing enzyme family protein, sugar ABC transporter permease, carbohydrate ABC transporter permease, gfo/Idh/MocA family oxidoreductase, NAD(P)-dependent oxidoreductase, M24 family metallopeptidase, sugar phosphate isomerase/epimerase, dihydrodipicolinate synthase family protein, carbohydrate ABC transporter substrate-binding protein
G. halophytocola KLMB5180	MI10	N-acetyltransferase, AraC family transcriptional regulator, ABC transporter ATP-binding protein, iron-siderophore ABC transporter substrate-binding protein, iron ABC transporter permease, hypothetical protein, ROK family protein, extracellular solute-binding protein, sugar ABC transporter permease, arbohydrate ABC transporter permease, alpha-amylase, FAD-dependent oxidoreductase, APC family permease, TetR/AcrR family transcriptional regulator
G. halophytocola KLMB5180	MI13	sugar ABC transporter substrate-binding protein, antibiotic biosynthesis monooxygenase, galE UDP-glucose 4-epimerase, galK galactokinase, galT galactose-1-phosphate uridylyltransferase, DeoR/GlpR transcriptional regulator, alpha-galactosidase, sugar ABC transporter permease, carbohydrate ABC transporter permease, sugar ABC transporter substrate-binding protein

of which SI1 displayed proteins involved in transmembrane transportation and transcriptional regulation, both of which are important to virulence and soil adaptation. (Table 3), (Zhang et al., 2017). For *G. nicotinae* OCT16, the metabolic islands MI1 and SI3 were reported as exclusive genomic islands (Supplementary Table 7; Supplementary figure 6), these two islands display genes encoding for proteins involved in sugar transportation, oxidative stress and transcriptional regulation,

all of which are important in adaptation to soil environments (Table 4). Strains YCRL1 and OTC16 were isolated from soil, but belonged to different soils, which is why islands were predicted individually. In addition, these 2 strains, as well as strain RE117 (isolated from cheese) had the largest number of shared genomic islands.

The species *G. halophytocola* KLMB5180 had 18 exclusive genomic islands (Supplementary Table 8; Supplementary figure 7). Of these 18

GEIs, 2 are symbiotic islands, 5 pathogenic, and 7 metabolic. Islands MI1, MI10 and MI13 display genes involved in transcriptional regulation, sugar transportation, and iron and amino acid metabolism (Table 4). Genomic islands functionally involved in niche adaptation were reported, but genes involved in specialization were not found, which could be due to the fact that specialization genes cannot be transferred through HGT (Bonham et al., 2017). In addition, strains isolated from specific niches are likely not niche restricted, having an ample niche adaptation spectrum (Feng et al., 2017).

In this experiment 126 GEIs were predicted, of which 67 are homologous between one or more genomes. In the results, there were only 2 GEIs present in all genomes, suggesting that these 2 GEIs may be a characteristic of the genus. The pathogenicity-related islands accounted for 43.30% (55 in total), MIs accounted for 5.19% (32 in total), RIs accounted for 18.89% (24 in total) and SIs accounted for 11.8% (15 in total).

3.6. Antibiotic and bioremediation resistance genes in G. creatinolyticus

The search of the resistance and virulence repertoire shared between genomes, was facilitated by BLASTX sequence comparison to the MEGARes: An Antimicrobial Database for High-Throughput Sequencing database (Lakin et al., 2017) (percentages of identity with the matching regions are shown in the parenthesis).

The genome of the *G. cretinolyticus* LGCM 259 displayed various antibiotic resistance genes. The rifamycin-resistant beta-subunit of RNA polymerase (rpoB) (77%) gene was identified, a marker that includes amino acid substitutions which disrupt the affinity of rifampin for its binding site (Miller et al., 1994). Elfamycin EF-Tu_inhibition (75%) was also detected. The *tufA* and *tufB* genes encode the components of EF-Tu, the target of the elfamycins. Mutations in them can cause elfamycin resistance via evasion of the mechanism of action (Vorstenbosch et al., 2000). Among the other identified genes are Aminocoumarin (100%) resistance genes. Aminocoumarin acts through the inhibition of the DNA-gyrase enzyme involved in bacterial cell division (Flatman et al., 2005), and the ABC transporter (100%) gene, one of the most widely recognized mechanisms of multidrug resistance, which can be considered a hijacking of their normal roles in the transport of xenobiotics, metabolites and signaling molecules across cell membranes (Fletcher et al., 2016). Besides, the genome of strain LGCM 259 presents arsenic resistance genes, as the *arsC* and *arsB* genes. ArsC codes for a small-molecular mass arsenate reductase, and ArsB codes an arsenic efflux pump protein which forms an anion-translocating ATPase (Cai et al., 2009). Moreover, heavy metal resistance genes (related to copper, arsenic, chromium, and cobalt-zinc-cadmium resistance) were identified.

Interestingly, only Rifampin resistance is shared between all genomes. Other antibiotic resistances are present in one or more genomes (Fig. 5). Only strain LGCM 259 displayed chloramphenicol resistance. Elfamycin resistance is shared only by strains KLBM5180 and YCRL1. Fluoroquinolone resistance was present in nearly all genomes, except for strain KLBM5180. Aminocoumarin resistance was present in strains LGCM 259, KLBM5180, and OTC-16. Spiramycin resistance could only be detected in the OTC-16 genome. The *A. sp.* YCRL1genome displayed a gene conferring Multi-drug resistance. The ABC transporter resistance gene was present only in strains LGCM 259 and YCRL1.

4. Discussion and conclusion

The bacteria that make up the *Glutamicibacter* genus are aerobic, Gram-positive, and metabolically diverse bacteria that are broadly distributed between soil, human and, in this case, as an occasional pathogen associated to infections (Hou et al., 1998). In general, this study seeks to better our comprehension of the genomic resources of *G. Creatinolyticus* LGCM259 that may be employed in order to resist and survive in a habitat unlike those previously described for the genus.

Bacterial virulence factors allow for a bacterium to replicate and

multiply within a host (Cross, 2008). The *G. creatinolyticus* LGCM 259 genome has displayed virulence factors associated with an increase in virulence, probably influencing the bacterium's capacity to infect, and survive within, a host, as well as virulence factors essential to cellular viability and pathogenicity (Muñoz-Elías and McKinney, 2005; Wall et al., 2005).

Genomes share many common islands, but some of them are unique to each species, suggesting that these unique GEIs may be related to the adaptation of each species, but if these islands are unique for each strain or if it is species related, further studies are needed involving more representatives of each species. The *G. creatinolyticus* LGCM 259 genome is smaller than that of the other four species used in this work, due to the loss of genomic regions. This functional loss is likely tied to the adaptation of *G. creatinolyticus LGCM 259* to its specialized environment, or animal host (mare). Typically, when a bacterium is adapted to infecting a host, there is a reduction of its genome size, since environmental species need genes encoding environmental stress resistance, such as to extreme temperatures, pH, and high salinity (Krasensky and Jonak, 2012; Toft and Andersson, 2010).

The genome displayed a broad repertoire of antimicrobial resistance genes. The emergence of antimicrobial resistant bacteria has become a major public health issue, due to the lack of effective antimicrobial agents available for the treatment of bacteria-caused diseases (Aslam et al., 2018). We also observed that the 4 genomes were resistant to multiple antibiotic classes, such as Rifampin, Elfamycin and Fluoroquinolone. Multiple drug efflux mechanisms contribute significantly to intrinsic and acquired antimicrobial agent resistance. The following locus tags were found (GcLGCM259_1698, GcLGCM259_0905 and GcLGCM259_1698) in the LGCM 259 genome, which may be involved in multiple drug resistance. Among resistance mechanisms, the multiple drug efflux system, or pump system, is deserving of special attention, since it allows the bacterium to reduce, or even nullify, its susceptibility to a broad range of antimicrobial agents (Moreira et al., 2004). According to the World Health Organization, antimicrobial resistance is a complex problem that affects all of society and is driven by many interconnected factors. Single isolated interventions have limited impact. Coordinated action is required to minimize the emergence and spread of antimicrobial resistance (World Health Organization, 2018).

According to Prum, 2018, *G. creatinolyticus* has proven to be efficient in the decontamination of arsenic contaminated water, through the interaction between plants and bacteria (Prum et al., 2018). That is because it can tolerate higher levels of arsenic toxicity, as well as its capacity to produce higher levels of indol-3-acetic acid (IAA) and ammonia than *Bacillus subtilis* (Prum et al., 2018). In other works, *G. creatinolyticus* has proven to be a potent producer of extracellular urease for the determination of heavy metal ions (Ramesh et al., 2014) and
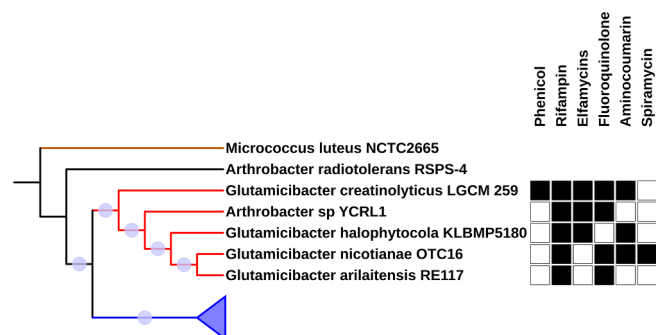

Fig. 5. Cluster of the genus *Glutamicibacter*, together with an f the genus A*rthrobacter* species including information on the presence of resistance classes of genes. The cluster is highlighted in red and black squares represent gene presence of the resistance class. Purple balls represent a bootstrap of 100%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

been deployed as a potentiometric biosensor for the determination of urea content in milk, using mobilized urease (Ramesh et al., 2015). Heavy metals are very useful, due to being used in the manufacture of tools, such as pipes and batteries, but are also among the most well-known toxic substances. However, some bacteria isolated from various sources have demonstrated a capacity to survive in high concentration of toxic heavy metals (Castro-Silva et al., 2003; Pontes et al., 2007).

G. creatinolyticus has displayed genes that may help confer it resistance to heavy metals such as: arsenic, cobalt-zinc-cadmium (*cobT*, *cobS*, *cadA*), and chrome composts (locus tag GcLGCM259_2553 – Chromate efflux transporter ChrA), which are serious environmental contaminants. Species such as *Klebsiella pneumoniae* MS 1.5 and *Mangrovibacter yixingensis* strain MS 2.4 have displayed a high capacity to reduce chrome, and because of it, may be useful for the decontamination of chrome contaminated environments (Sanjay et al., 2018). The genome also displayed copper tolerance genes (*copZ*, *csoR_1*, *cutC*, *aniA*, *pcoC*), copper is an essential element amongst heavy metals, which plays a major role in the growth and physiology of aerobic organisms, however, this metal can cause cellular death when in excess. In the species *Cupriavidus metallidurans* CH34, *Pseudomonas syringae* pv. *tomato* PT23, *Xanthomonas axonopodis* pv. *Vesicatoria* E3C5 and *Pseudomonas aeruginosa* PAO1, the *cop* genes have been identified (*copA*, *copB*, *copC* e *copD*) which confer copper resistance (Altimira et al., 2012). Some microorganisms are responsible for environmental transformations of the metal, and therefore may be useful as indicators for assays both in polluted and non-polluted environments (Castro-Silva et al., 2003; Trevors et al., 1985). This implies there may be biotechnological applications for metal resistant bacteria in the control of toxic metals in residual water treatment (Sanjay et al., 2018). These discoveries are relevant for the mining industry, as they pertain to bioremediation (Sanjay et al., 2018), however, additional studies are necessary, in order to better understand and explore this bacterium's characteristics and metabolic mechanisms.

In a previous study's regarding the identification of these species, authors report that there are difficulties in identifying these species of clinical samples by conventional biochemical tests because their reliability at the species level is limited (Yamamoto et al., 2017). Infections due to this species may have been underestimated because a correct identification of *G. creatinolyticus* is only possible by applying further identification methods (i.e., 16S rRNA gene sequencing or MALDI-TOF MS) (Funke et al., 1996; Yamamoto et al., 2017).

Finally, our results provide important new information about the genetic background of a strain of *G. creatinolyticus*. Comparative genomic analyses between the genome of *G. creatinolyticus* LGCM 259 and other genomes of *Glutamicibacter* and an *Arthrobacter* revealed the genetic mechanisms of its virulence, as well as genetic reductions, probably responsible for the adaptation to the host. Genomic islands displayed many genes vital to each species adaptation to their specific niche, such as oxidative stress, and transcriptional regulation. The genome also displays various genes which may be related to heavy metal resistance. However, more studies are needed to evaluate the importance and distribution of this species in its various habitats.

Author contributions

Wrote the manuscript (RS, NS, TC, LG, RH); designed the study (RS, NS, TC, AR, RC); experimental work (RS, NS, TC and LG); conducted in silico analyses and interpreted the results (RS, RP, NS, RH); critically reviewed and revised the manuscript (NS, FR, CV, SJ, MG, AG, AG, RP, RH, and AR); supervised the study (NS, VA). Funding (BB, VA and NS.). All authors approved this manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2020.144566.

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