Investigation of islands of resistance in complete genomes of *Brucella abortus*

https://doi.org/10.56238/sevened2024.026-033

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ABSTRACT

The current evolution of sequencing platforms has increased the amount of information generated and drastically reduced prices. The use of these technologies enabled the development of several omics sciences. Thus, conducting in *silico experiments* through bioinformatics provides basic information for various omics analyses, such as genomics, transcriptomics, metabolomics, among others.

These analyses are especially important when the target of the study is a microorganism of biotechnological, agricultural or environmental interest, as is the case of *Brucella abortus*, a Gram-negative, immobile bacterium with absence of flagella, non-capsulated, non-spore-forming, aerobic, considered a facultative intracellular pathogen and whose cultivation is considered complex.

Thus, the objective of this project is to analyze the complete genomes of *Brucella abortus* available in databases, through bioinformatics approaches, to characterize the resistance profile, evolution and mechanisms of virulence and pathogenicity.

Keywords: *Brucella abortus*, Bioinformatics, Genomics, NGS, Prokaryotes.

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INTRODUCTION

The set of antibiotic resistance genes of microorganisms is called the resistome, proposed by Gerard D. Wright (Wright, 2007). The resistome encompasses the resistance genes of pathogenic and non-pathogenic bacteria, which include antibiotic-producing genes and genes that encode proteins with modest resistance or with binding capacity to antibiotic molecules, inhibiting their action (Caumo et al., 2010).

The incorrect disposal and indiscriminate use of antibiotics are factors that have contributed to the increase in bacteria resistant to various classes of antibiotics. In the past, antibiotic resistance was restricted to hospital settings, but more recent studies have demonstrated the presence of these resistant bacteria in various environments, such as water and soil (Alves et al., 2020). Contamination of the environment by antibiotics can occur through waste from hospital units, agriculture, livestock and/or the disposal of human waste in inappropriate places. The presence of these antibiotics in these environments acts by naturally selecting resistant bacteria, and these in turn can transmit resistance genes through plasmids, integrons, and transposons, altering the balance of the microbial environment (Andersson et al., 2016).

Horizontal gene transfer plays an important role in the development and dissemination of antibiotic-resistant bacteria through the acquisition of resistance genes, conferring new metabolic capacities to the microorganism that receives exogenous DNA, allowing its survival and proliferation in antibiotic-present environments (Alonso et al., 2001).

According to Baba et al (2002), genomic islands (GIs) that are resistant to antibiotics are called islands of resistance (REIs). According to Yoon et al (2015), REIs are a class of GIs linked to pathogenesis, conferring simultaneous resistance to multiple antibiotics and facilitating the emergence of multidrug-resistant pathogens.

Thus, the investigation of resistance genes in organisms of biotechnological and medical-veterinary interest are extremely important, such as the microorganism with which brucellosis is caused in cattle. *Brucella abortus* is a gram-negative bacterium that can cause brucellosis in cattle, leading to miscarriage in pregnant cows, and infertility in male cattle (Neta et al., 2010). Bovine brucellosis is a disease responsible for causing major economic impacts in the agricultural sector of cattle production, mainly causing abortions in the final stretch of pregnancy and the birth of weakened calves, which can cause the early disposal of the animal, losses that justify efforts in the search for effective control solutions, which has vaccination as its most important strategy (Souza, 2009).

METHODOLOGY

DATA COLLECTION

Twenty-three (23) complete genomes of *Brucella abortus* (Table 1) and one of *Escherichia coli* str. K-12 substr. MG1655 deposited in the National Center for Biotechnology Information (NCBI) public database.

Table 1. Complete strains of Brucella abortus available at NCBI.

STRAINS	BioSample	BioProject	Identification	SUBMISSION	REGISTRATION DATE
CIIMS-NV-4	SAMN02604294	PRJNA18999	NC_010742.1	Dr. GM Taori, Central India Institute of Medical Sciences	January 22, 2018
A19	SAMN02604220	PRJNA9619	NC_006932.1	Inner Mongolia Agricultural University	July 8, 2018
NCTC 10505	SAMN02603577	PRJNA74681	NC_016795.1	Los Alamos National Laboratory	August 15, 2014
19BA	SAMN08295219	PRJNA428582	NZ_CP025743.1	RARI and FSBI SCEEMP	June 18, 2018
clpP	SAMN09499628	PRJNA478292	NZ_CP030751.1	China Animal Disease Control Center	October 1, 2019
BAB8416	SAMN02769901	PRJNA244264	NZ_CP007700.1	Shanghai Jiao Tong University School of Medicine	June 30, 2015
BD	SAMN09405586	PRJNA475940	NZ_CP077765.1	China Agricultural University	August 22, 2017
MC	SAMN12827000	PRJNA573837	NZ_CP044338.1	China Agricultural University	August 22, 2017
BJ1	SAMN02712083	PRJNA243017	NZ_CP008774.1	China Institute of Veterinary Drug Control	October 23, 2018
68	SAMN07508059	PRJNA398311	NZ_CP022877.1	University of Alaska Fairbanks	December 17, 2020
BDW	SAMN07508100	PRJNA398313	1:NZ_CP022879.1	Los Alamos National Laboratory	August 15, 2014
ABOUT	SAMN10238131	PRJNA496294	1:NZ_CP033079.1	Los Alamos National Laboratory	August 15, 2014
BFY	SAMN12837785	PRJNA573988	I:NZ_CP046720.1	Los Alamos National Laboratory	August 15, 2014
63 75	SAMN02769911	PRJNA243877	1:NZ_CP007705.1	Los Alamos National Laboratory	August 15, 2014
104M	SAMN02770262	PRJNA244260	1:NZ_CP007709.1	Beijing Institute of Biotechnology	September 22, 2015
2308	SAMN02770266	PRJNA243881	1:NZ_CP007765.1	Oak Ridge National Laboratory	November 28, 2005
A13334	SAMN03108762	PRJNA263969	1:NZ_CP009625.1	Macrogen	15 December 2011
RB51	SAMN02767917	PRJNA243872	1:NZ_CP007681.1	U.S. Department of Agriculture	March 31, 2020

S19	SAMN02767934	PRJNA243875	1:NZ_CP007682.1	Virginia Bioinformatics Institute	May 22nd, 2008
9-941	SAMN02786918	PRJNA244261	1:NZ_CP007738.1	University of Minnesota	April 5, 2005
86/8/59	SAMN02767544	PRJNA243873	1:NZ_CP007663.1	Los Alamos National Laboratory	August 15, 2014
870	SAMN17078505	PRJNA685163	1:CP066175.1	Los Alamos National Laboratory	August 15, 2014
C68	SAMEA3138256	PRJNA16203	I:NC_007618.1	Los Alamos National Laboratory	August 15, 2014

Source: NCBI (2022).

PHYLOGENETIC ANALYSIS

The phylogenetic tree will be performed from the *rpoB gene*, and the multiple alignment will be performed by the BioEdit program (Alzohairy, 2011), through the ClustalW program, and the construction of the tree by the MEGAX program (Kumar et al., 2018), using the Maximum Parsimony method with 1000 bootstrap replicates. As an external group, the genome used will be that of the *Escherichia coli* str strain . K-12 substr. MG1655 available in the NCBI database under access number NC_000913.3.

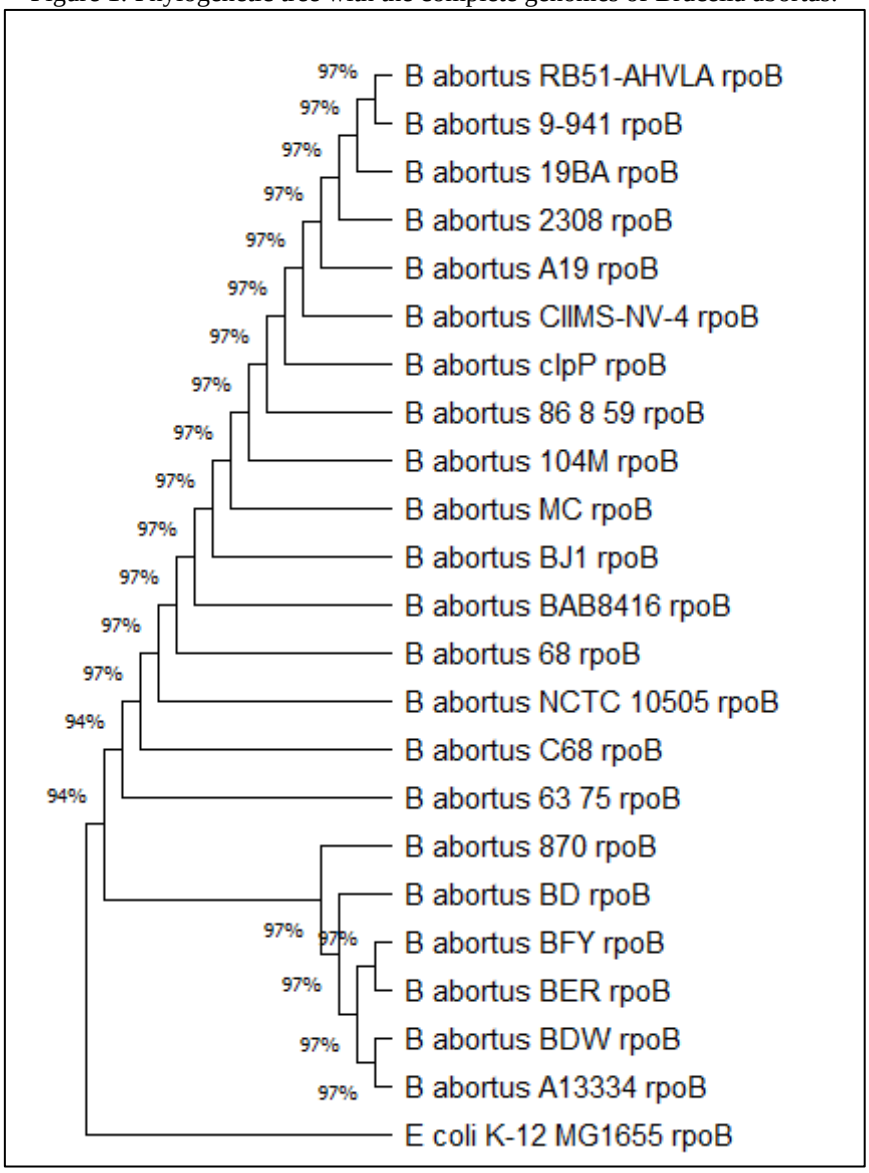
ISLANDS OF RESISTANCE

The identification of resistance islands will be carried out by the Gipsy program (Soares et al., 2016), and this tool is responsible for predicting genomic characteristics shared between a pathogenic and a non-pathogenic species. The standard parameters of the program will be adopted for GA prediction: GC 1.5 content deviation, codon 0.95 use deviation, transposase genes 1E-04, search for specific factors 1E-06, tRNA genes 1E-04 and absence in other organisms 1E-06.

RESULTS

The phylogenetic tree was performed with 23 strains of *B. abortus*, represented by figure 1, in which bootstrap values of at least 94% can be observed, evidencing a high rate of proximity between the strains.

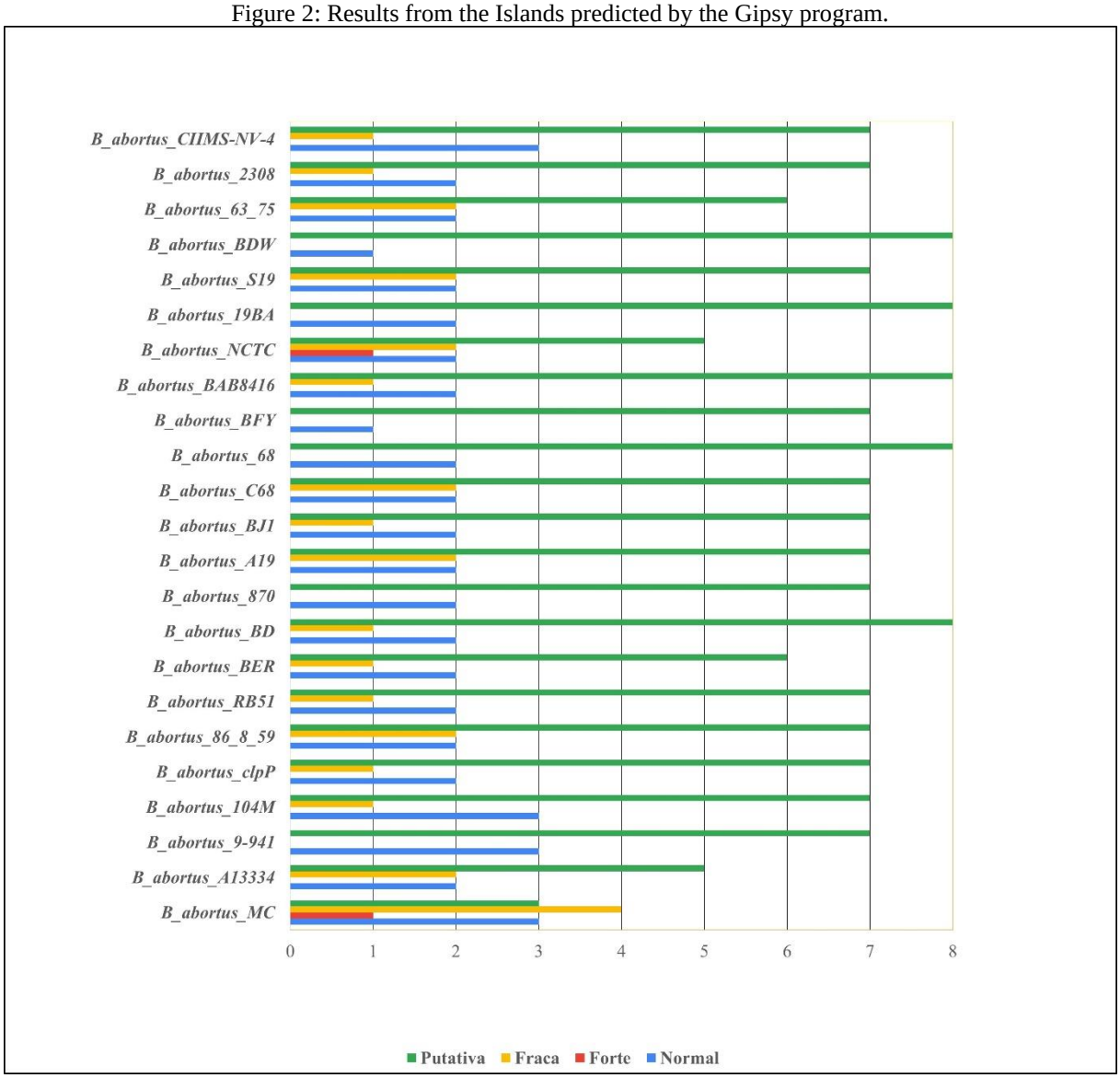
Figure 1: Phylogenetic tree with the complete genomes of Brucella abortus.



After the creation of the phylogenetic tree, the identification of the resistance islands of the Gipsy program was carried out.

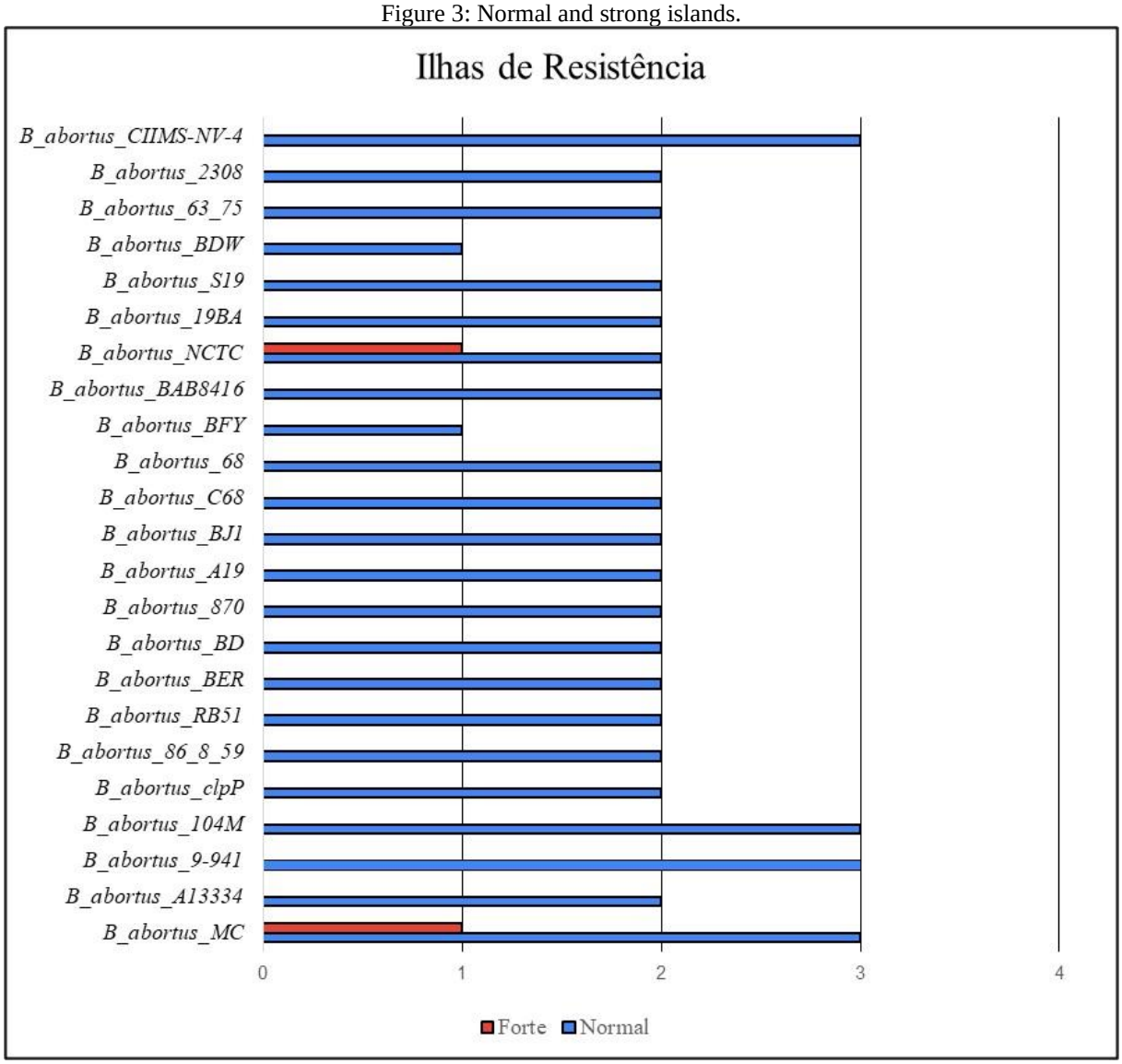
Figure 2 shows the number of REIs found in B. *abortus* strains, which are classified as putative (156), weak (27), strong (2) and normal (48). The number of REIs found is 233.

Figure 2: Results from the Islands predicted by the Gipsy program.



The results of interests are normal and strong presented in figure 3. Strains with the highest numbers of identified islands B . *abortus MC* (4), *B. abortus 9-941* (3), *B. abortus 104M* (3), *B. abortus CIIMS-NV-4* (3).

Figure 3: Normal and strong islands.



DISCUSSION

According to Moreira et al (2015), the phylogenetic tree is a graphic representation, presenting the evolutionary relationships between various species, genes, proteins or any other elements that may have a common ancestor.

The high value of similarity found between the strains in the phylogenetic tree (figure 1) was already expected, as they are strains of the same species and *the rpoB* analysis was performed. The *rpoB* gene (RNA polymerase beta subunit) is a highly conserved molecular marker, also used to identify bacteria and to study their evolutionary relationships (Pulingam, 2022).

Through the bootstrap method, which consists of a number associated with a certain clade in the tree present in the different databases (Efron, 1992). The phylogenetic tree (figure 1) showed high similarity between *B. abortus strains*.

According to Soares et al (2016), genomic islands receive a prediction score of "Weak", "Normal" or "Strong" according to the following rules:

I - Strong, these are regions that have very high concentrations of the specific factor chosen and all other characteristics when compared to the entire genome sequence;
II - Strong, also for regions that manifest low and normal concentration of one genomic signature trait, high concentration of the other and very high concentration of the specific factor chosen;
III - regions that have a low concentration of a
genomic signature trait and high concentration of the other, with a high concentration of the specific factor chosen.
IV - As well as the classification of normal, it is also used to classify regions with normal to high concentration of the specific factor chosen and also high concentrations of 36 hypothetical proteins and deviation of genomic signature; and, (V) otherwise weak (Soares et al., 2016).

The predictions of the islands of interest through GIPSy resulted in a total of 50 REIs identified. Among the results, only two strains showed strong REIs, the *B. abortus NCTC strain and B. abortus MC*, which indicates that these results have a high probability of being true IGEs, as they contain known resistance or pathogenicity genes (Soares et al., 2016).

CONCLUSION

In this study, it was found that the 23 strains analyzed have a great phylogenetic similarity. Using GIPSy to do some analysis and you notice some interesting changes. For example, the "G+C shift", which is basically a change in the percentage of Guanine and Cytosine in the genome. There is also a "codon use deviation", which refers to how often different combinations of nucleotides form an amino acid in protein synthesis.

Therefore, the results show that REIs may be related to pathogenic traits that may lead to the development of new antibiotic-resistant strains or that may cause new diseases. The study of genomic islands is important to understand the evolution of bacteria and the development of antibiotic resistance and the development of resistance islands to develop more efficient treatments against bacteria with pathogenic characteristics.

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