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Whole-Genome Sequence Analysis of the Naturally Competent *Acinetobacter baumannii* Clinical Isolate A118

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Abstract

Recent studies have demonstrated a high genomic plasticity in *Acinetobacter baumannii*, which may explain its high capacity to acquire multiple antibiotic resistance determinants and to survive in the hospital environment. *Acinetobacter baumannii* strain A118 (*Ab* A118) was isolated in the year 1995 from a blood culture of an intensive care unit patient. As this particular strain showed some peculiar characteristic such as being naturally competent and susceptible to numerous antibiotics, we performed whole-genome comparison (WGC) studies to gain insights into the nature and extent of the genomic differences. The *Ab* A118 genome is approximately 3,824 kb long with a 38.4% GC content and contains 3,520 coding sequences. WGC studies showed that the *Ab* A118 genome has 98% average nucleotide identity with that of *A. baumannii* ATCC 17978, and 96% average nucleotide identity with that of strains AYE and ACICU. At least 12 inversions, 275 insertions, and 626 deletions were identified when the *Ab* A118 genome was compared with those of strains ATCC 17978, AYE, and ACICU using MAUVE WGC. Multiple gene order arrangements were observed among the analyzed strains. MAUVE WGC analysis identified 19 conserved segments, known as locally colinear blocks. The number of single nucleotide polymorphisms found when comparing the *Ab* A118 genome with that of strains ATCC 17978, AYE, and ACICU was 43,784 (1.1496%), 44,130 (1.158%), and 43,914 (1.153%), respectively. Genes *comEA, pilQ, pilD, pilF, comL, pilA, comEC, pilI, pilH, pilO, pilN, pilY1(comC), pilE, pilR,* and *comM*, potentially involved in natural competence were found in the *Ab* A118 genome. In particular, unlike in most strains where *comM* is interrupted by an insertion of a resistance island (AbaR), in strain *Ab* A118 it is uninterrupted.

Key words: *Acinetobacter baumannii*, genome analysis, SNPs.

Introduction

Acinetobacter baumannii was recently recognized as a successful nosocomial pathogen, with an increasing morbidity and mortality due the rise in multi- and pan-drug-resistant strains (Perez et al. 2007). Its clinical importance led to extensive studies on different aspects of the biology and pathogenesis (McConnell et al. 2013). To this date, the genomes of 16 *A. baumannii* strains have been sequenced and those of more than 200 other isolates are as draft stage in GenBank. Genome comparisons showed high variability, which could be the result of the combination of natural competency and the presence of active recombination system(s) (Smith et al. 2007; Ramirez et al. 2011). These processes could also explain the unusual tendency of *A. baumannii* to acquire multiple antibiotic resistance determinants and to survive in the hospital environment (Hornsey et al. 2011; Snitkin et al. 2011; Sahl et al. 2013; Tan et al. 2013).

A recent study showed that three strains belonging to the international clonal lineage 2 (ICL2) have an elevated number of single nucleotide polymorphisms (SNPs) when compared with the ICL2 prototype ACICU strain. Most SNPs were preferentially located in specific "recombinant regions," which was interpreted as the product of homologous recombination-mediated DNA swapping (Snitkin et al. 2011). Taking into account these results, a comparison of the genomes of a tigecycline resistant with a susceptible strain isolated from a patient before and after 1-week treatment showed significant differences in addition to a single nucleotide mutation in the *adeS* gene that accounts for resistance to the antibiotic (Hornsey et al. 2011). Conversely, another comparative study between two *A. baumannii* strains, one of them an

extreme-drug resistant and the other a pan-drug resistant, showed only 61 SNPs between them (Tan et al. 2013). However, despite the relatively small number of SNPs observed between the two strains, the authors concluded that the changes are indicative of fast evolution because the mutations occurred within a 1-month period (Tan et al. 2013).

We recently initiated the characterization of the first laboratory confirmed naturally competent *A. baumannii* strain, *Ab* A118, an isolate that unlike other clinical isolates is susceptible to numerous antibiotics, supports replication and stable maintenance of different plasmid replicons and took up fluorophore labeled oligonucleotides (Ramirez et al. 2010, 2011). We considered that these characteristics make *Ab* A118 a convenient model for genetic studies. *Ab* A118 was isolated in the year 1995 in Buenos Aires City, Argentina, from a blood culture of an intensive care unit patient and did not belong to any of the predominant clonal complexes widespread in our region and in the world. Interestingly, although the *comM* gene is interrupted by the insertion of the AbaR resistance island in numerous strains, it is intact in *Ab* A118, a characteristic that could account for the susceptible phenotype and natural competency exhibited by this isolate (Ramirez et al. 2010, 2011). In this report, a comparative study to gain insight into the nature and extent of the genomic differences found in *Ab* A118 strain when its genome is compared with those of strains ATCC 17978, AYE, and ACICU is described.

Materials and Methods

The available scaffolds (accession number AEOW01000000) were ordered and oriented with the MAUVE Contig Mover (Darling et al. 2010), using the ATCC 17978 genome as reference (Smith et al. 2007). Genomes were aligned with the open-source MAUVE aligner version 2.3.1 using the progressive algorithm. The alignments were generated using the default settings (http://gel.ahabs.wisc.edu/mauve/, last accessed August 29, 2014). Coding sequence predictions and annotations were made by Rast version 4.0 and Glimmer2 software (Delcher et al. 1999; Aziz et al. 2008). tRNA genes were identified using tRNAscan-SE (Lowe and Eddy 1997). SNPs were displayed using the Circos software (http://circos.ca, last accessed August 29, 2014).

The *Ab* A118 genome is 3,824 kbp long (Ramirez et al. 2011) has an average GC content of 38.4%, and 88 tRNA genes and 3,520 coding sequences were identified, of which 93.64% could be annotated with high confidence and also manually curated using FASTA and BLAST results. Figure 1, obtained using Circos software, shows a diagram of the *Ab* A118 genome with the annotated genes in color codes according to Clusters of Orthologous Group (COG) category.

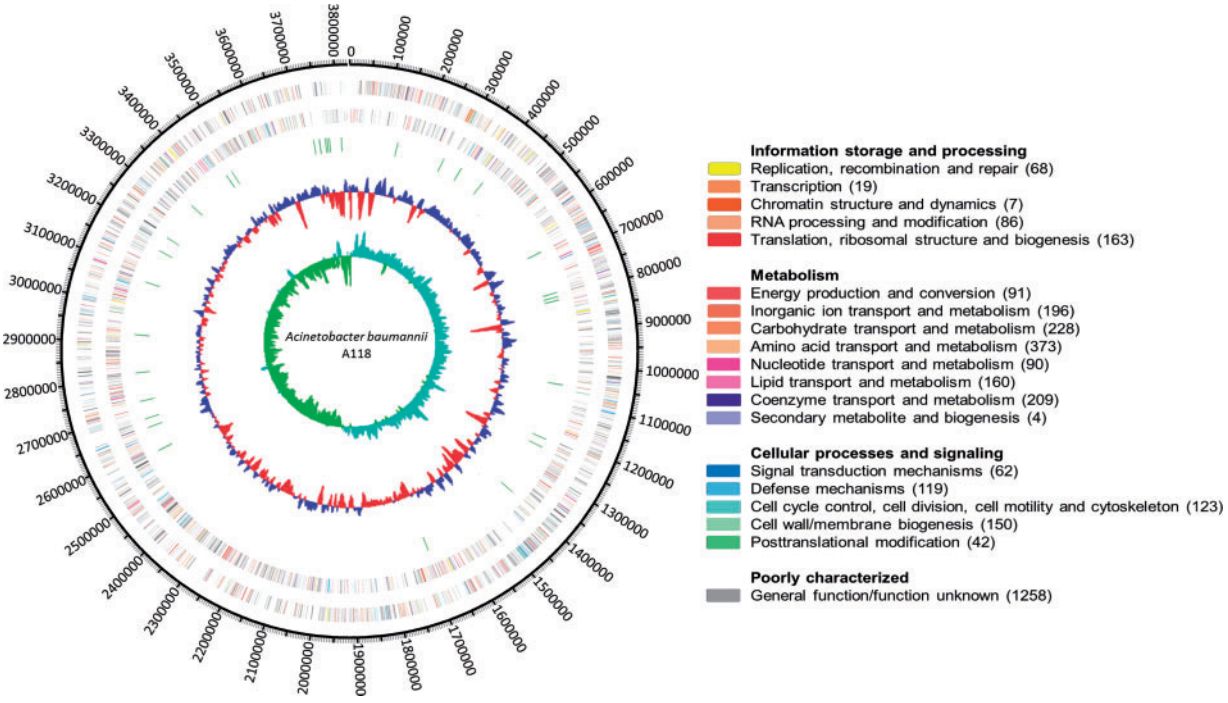

Fig. 1.—Circular genome representation of *Ab* A118. Rings from outside to inside: Ring 1: nucleotide coordinates in base pairs. Ring 2: open reading frame (ORF) distribution, plus strand. Ring 3: ORF distribution, negative strand. Ring 4: tRNA genes distribution represented in green lines. Ring 5: GC content, blue represents the above average content and red the bellow average content. Ring 6: GC skew, calculated in Artemis. ORFs are color coded based on COG classifications.

Comparative Analysis against Sequenced and Annotated *A. baumannii* Genomes

The phylogenetic tree diagram obtained using MAUVE and SplitsTree4 showed that ATCC 17978 strain is the most closely related strain to *Ab* A118 (supplementary fig. S1, Supplementary Material online). The *Ab* A118 genome showed 98% average nucleotide identity with that of ATCC 17978 (accession number CP000521), and 96% with those of AYE (accession number NC_010410) and ACICU (accession number NC_010611). These latter strains are representatives of the most widespread clonal complexes 1 and 2.

Gene order comparison using MAUVE alignment showed multiple changes and 19 regions with high homology named Local Colinear Blocks (LCBs), which encompassed an average of 81.73% of each genome (fig. 2). Table 1 shows the numbers of inversions, insertions, and deletions identified when comparing the *Ab* A118 genome with those of strains ATCC 17978, AYE, and ACICU. The results show that the ACICU strain has the small number of arrangements compared with *Ab* A118.

These genomic variations support the existing idea that *A. baumannii* has a distinct ability to exchange genetic material and rearrange its genome, a property that must impact its ability to adapt to hostile environments.

Comparison of SNPs Distribution across *A. baumannii* Strains

The number of SNPs presented by the *Ab* A118 genome in comparison with all three other strains is shown in table 1 and figure 3. The number of SNPs identified between *Ab* A118 and ATCC 17978, AYE, and ACICU strains was 43,784 (1.1496%), 44,130 (1.158%), and 43,914 (1.153%), respectively (fig. 3 and table 1). The SNPs were located in 2,571 genes (73%), 2,791 genes (79%), and 2,882 genes (82%) with respect to the genomes of ATCC 17978, AYE, and ACICU, respectively. These genes are predicted to be involved in core biological functions, such as metabolism, replication, transcription, natural competence, antibiotic resistance, cytoskeleton, and others.

Table 1

Genome Rearrangements and SNPs Obtained from the Comparison of *Ab* 118 Genome to Those of Strains ATCC 17978, AYE, and ACICU

Strain	Inversions	Insertions	Deletions	SNPs (%)
ATCC 17978	12	206	626+	43,784 (1.1496)
AYE	6	275	350	44,130 (1.158)
ACICU	5	201	601	43,914 (1.153)

Fig. 2.—Multiple alignment of *Ab* A118, ATCC 17978, AYE, and ACICU genomes. The nucleotide sequences of *Ab* A118 genome (accession number AEOW01000000), ATCC 17978 (accession number CP000521), AYE (accession number NC_010410), and ACICU (accession number NC_010611) were compared using the MAUVE aligner version 2.3.1 (Darling et al. 2010). The figure was generated by MAUVE viewer. LCBs are represented by blocks of different colors. The degree of similarity is indicated using white areas. The colored area is higher where the similarity is high. Conversely, areas of low similarity are identified by larger white portions. Areas that are completely white within an LCB are not aligned and probably contain sequence elements specific to a particular genome.

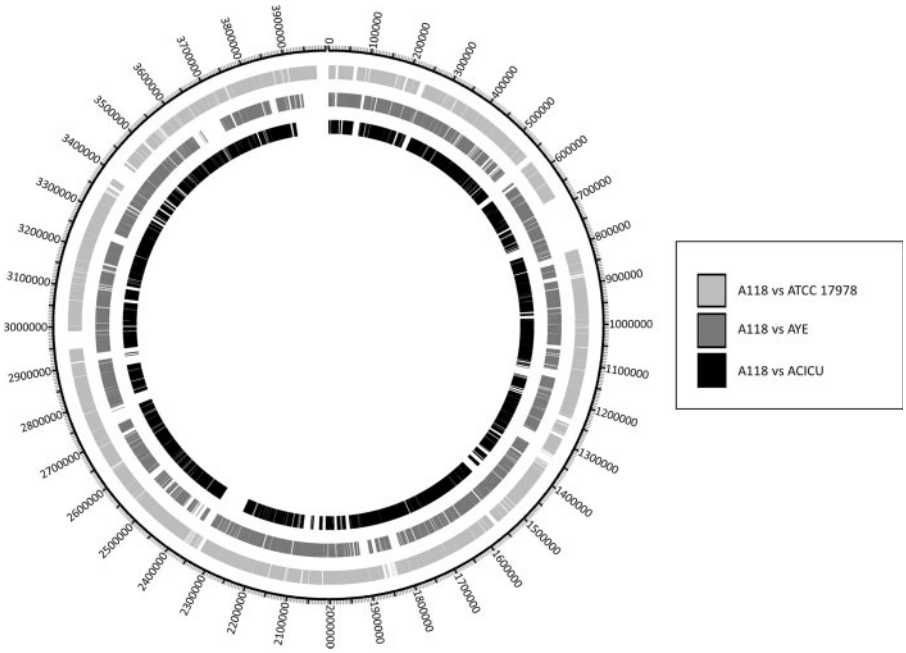

Fig. 3.—Distribution of SNPs among *Ab* 118, ATCC 17978, AYE, and ACICU genomes. SNPs in the genome alignments were determined by using Mauve and displayed with Circos. SNPs presence were filtered using the conditions described by Snitkin et al. (2011).

Table 2

SNPs and Amino Acid Variation Found Presumptive Competence Genes

Feature Name[a]	Coordinate in *Ab* A118	SNPs	Nonsynonymous Substitution/ Amino Acid
Pilin-like competence	3140807–3141067	16	0/0
Pilin-like competence—Tfp pilus assembly protein pile	3141224–3141652	4	4/4
Possible pilus assembly protein tip-associated adhesin pily1(comc)	3141719–3145564	48	30/30
Possible pilus assembly protein pilw	3146528–3147343	2	1/1
PilD-dependent protein pddC	2635560–2635940	12	0/0
PilD-dependent protein pddD	2636016–2636612	9	0/0
Putative competence protein (ComL)	1992614–1993402	6	0/0
Competence factor involved in DNA uptake—ComEC/Rec2	1041011–1043443	47	25/25
Putative membrane protein ComM	3618207–3619217	8	0/0
Putative membrane protein ComN	3617566–3618207	4	2/2
Putative membrane protein (ComO)	3616829–3617569	8	1/1
Putative outer membrane protein (ComQ)	3614076–3616241	22	1/1

[a]Feature name found in the *Acinetobacter baumannii* ATCC 17978 strain.

Apart from the remarkable difference with other strains for having an intact *comM* gene, all other presumptive competent-related genes in strain *Ab* A118 shared between 94% and 100% of amino acidic identity with those of all the complete annotated *A. baumannii* genomes. Analysis of the presence of SNPs in 12 presumptive competent-related genes when comparing strains *Ab* A118 and ATCC 17978 showed 186 SNPs with an average of 16 per gene (table 2). Most SNPs resulted in silent mutations or few amino acid substitutions (1–4). However, the SNPs found in the genes annotated as *pilY1* and *comEC/rec2*, which was recently reported as an important gene with a role in natural competence (Wilharm et al. 2013), resulted in 30 and 25 amino acid changes, respectively. The high variability among these genes and the presence of an intact *comM* gene may play a role in the natural competency. Functional and comparative studies are needed to determine the role of these genes in the mechanisms and efficiency of natural transformation in these strains.

Acinetobacter baumannii presents some remarkable survival features as its capacity to develop resistance to all antibiotics currently in use as well as its ability to withstand desiccation and resist most disinfectants. These traits, together with other characteristics such as the ability to form biofilms, provide *A. baumannii* strains the genetic armory to survive for long periods of time in the hospital environment. Its intrinsic genetic plasticity must be a key factor in the rapid adaptation necessary to survive the hostile environments in hospitals (Smith et al. 2007; Iacono et al. 2008; Peleg et al. 2008; Adams et al. 2009; Roca et al. 2012).

The *Ab* A118 genome includes a variety of genomic arrays with respect to others and includes a high number of SNPs consistent with high genomic variability. In particular, considering the previous finding that the *comM* gene is uninterrupted by an AbaR insertion (Ramirez et al. 2011), there is also a high variability in at least two other genes potentially involved in natural competency. This opens questions about the potential of this strain to behave differently than others with respect to acquisition of DNA from the environment. Comparative studies of the competency of several strains as well as mutagenesis analysis will contribute to the clarification of the mechanisms, efficiency, and roles of natural competency in *A. baumannii.*

Supplementary Material

Supplementary figure S1 is available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

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