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Genome-assisted identification of putative iron-utilization genes in *Acinetobacter baumannii* and their distribution among a genotypically diverse collection of clinical isolates

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Abstract

New putative iron-uptake genes were identified in published genomes of the opportunistic human pathogen *Acinetobacter baumannii*, and their occurrence was determined in a genotypically distinct collection of 50 clinical isolates by PCR and Southern blot assays. The results demonstrated that all *A. baumannii* isolates tested share the coding potential for two endogenous siderophores, a heme-acquisition and a ferrous iron-uptake system. A second heme-uptake cluster was detected in almost two thirds of isolates, without any apparent correlation with the clonal lineage of the strains. The wide distribution of multiple iron-acquisition systems among diverse *A. baumannii* clinical isolates argues for a contribution of iron uptake to the pathogenicity of this species.

Keywords: Acinetobactin; Feo system; Heme uptake; Siderophore

1. Introduction

Acinetobacter baumannii is the most clinically important species of the *Acinetobacter* genus and is generally found only in the hospital environment (Towner, 2009). *A. baumannii* has been widely studied for its ability to steadily acquire multiple antibiotic resistance genes (Dijkshoorn et al., 2007). However, the mechanisms which enable *A. baumannii* to colonize the human host and establish infection deserve further investigation.

One of the major challenges pathogenic bacteria face in their host is the scarcity of freely available iron. In mammals, iron is virtually unavailable to invading bacteria, being mainly incorporated into iron transport and storage proteins. The host's iron withholding capacity substantially reduces the bioavailability of iron to infecting bacteria and constitutes an important component of innate immunity (Guerinot, 1994). This is particularly important for *A. baumannii* opportunism, since this organism has to combat the iron-binding capacity of lactoferrin and transferrin during mucosal and bloodstream infections, respectively.

While iron withholding by the host resists establishment of an infection, pathogens respond by exploiting a number of strategies for high-affinity iron acquisition, including production of ferric [Fe(III)] iron chelators (siderophores), uptake of exogenous chelators, such as heme and heterologous siderophores, and acquisition of ferrous [Fe(II)] iron. Bacterial cells acquire Fe(III)-loaded siderophores and heme by means of specific receptor proteins. In Gram-negative bacteria, these receptors are localized on the outer membrane, where internalization of the siderophore-Fe(III) complex or heme is coupled to dissipation of the proton gradient on the inner membrane. Energy transduction from the inner to the outer membrane is mediated by the TonB protein complex in the periplasmic space (Miethke and Marahiel, 2007). Another major route for bacterial iron assimilation is the direct uptake

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of Fe(II) by the Feo system which, in γ-proteobacteria, consists of the cytosolic FeoA protein, the inner membrane Fe(II) permease FeoB, and the putative transcriptional repressor FeoC (Cartron et al., 2006).

The first iron-uptake molecule characterized in *A. baumannii* was a mixed catechol–hydroxamate siderophore termed acinetobactin (Yamamoto et al., 1994; Dorsey et al., 2004; Mihara et al., 2004). The recent publication of the complete genome sequences of six *A. baumannii* strains, namely ATCC 17978, AYE, SDF, ACICU, AB0057 and AB307-294, revealed that an acinetobactin gene cluster is present in all but one of the sequenced strains, being absent in the non-clinical isolate SDF (Adams et al., 2008; Iacono et al., 2008; Smith et al., 2007; Vallenet et al., 2008).

The aim of this study was to analyze the available *A. baumannii* genome sequences for additional gene clusters putatively involved in iron acquisition, and to establish the distribution of identified iron-uptake genes in a genotypically diverse collection of 50 clinical *A. baumannii* isolates, comprising representatives of the major epidemic worldwide lineages.

2. Materials and methods

2.1. Bacterial strains and growth media

The collection of *A. baumannii* strains used in this study includes the type strain ATCC 19606^T, the sequenced strains ACICU, ATCC 17978, AYE and SDF, and 50 genotypically diverse clinical isolates (see list in Fig. 1) collected as part of the EU-funded Antibiotic Resistance; Prevention and Control (ARPAC) project (MacKenzie et al., 2005; Towner et al.,

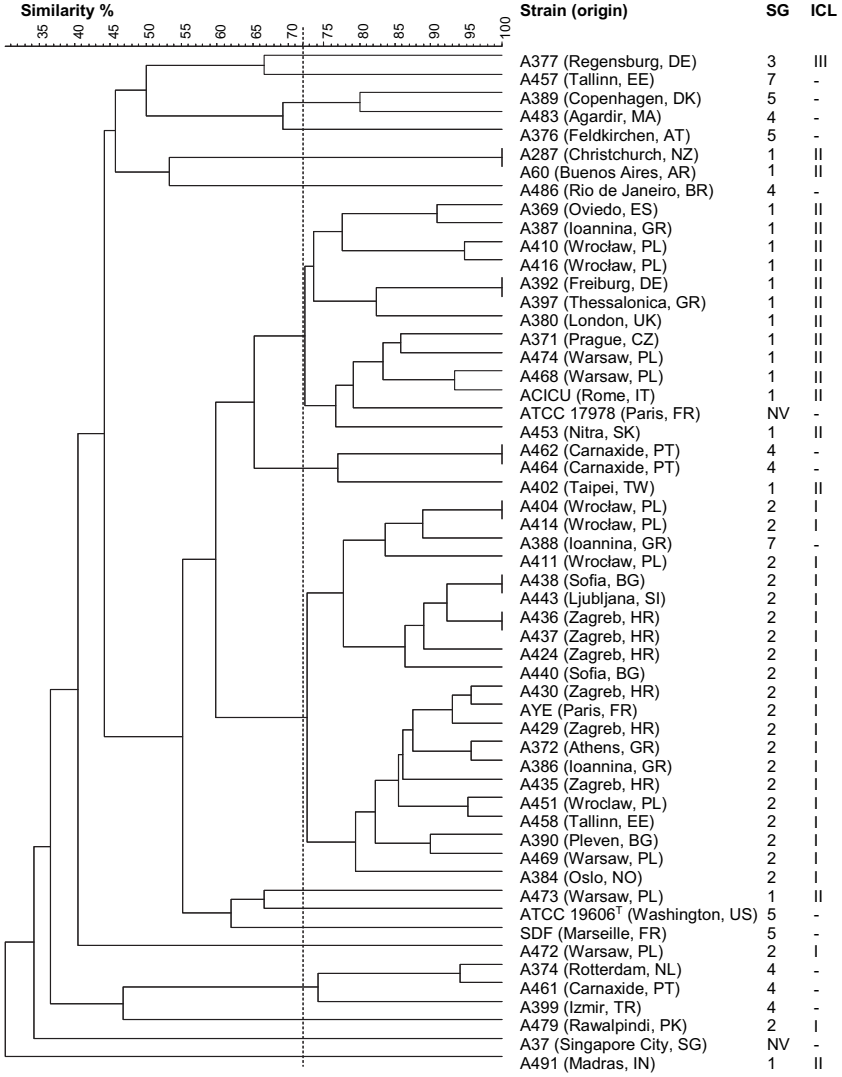

Fig. 1. Clustering relationships of the 50 clinical isolates, the type strain ATCC 19606^T and the four sequenced strains used in this study. RAPD analysis was performed with primer DAF-4. The dendrogram was generated with Bionumerics v.5.1 software (Applied Maths) using the Dice coefficient and the UPGMA method. The dotted vertical line defines the 72% cut-off level used previously to group isolates into the major epidemic lineages/clones. The sequence group (SG) and the origin of each strain are also shown. NV, new variant SG. As reported in figure, SG1, SG2 and SG3 correspond to the International clonal lineages (ICL) 2, 1 and 3, respectively (Turton et al., 2007). Two-letters country codes are according to the ISO standard ISO 3166-1 alpha-2: AR, Argentina; AT, Austria; BG, Bulgaria; BR, Brazil; CZ, Czech Republic; DE, Germany; DK, Denmark; EE, Estonia; ES, Spain; FR, France; GR, Greece; HR, Croatia; IN, India; IT, Italy; MA, Morocco; NL, The Netherlands; NO, Norway; NZ, New Zealand; PK, Pakistan; PL, Poland; PT, Portugal; SG, Singapore; SI, Slovenia; SK, Slovakia; TR, Turkey; TW, Taiwan; UK, United Kingdom; US, United States.