

Genomic and physiological characterization of a laboratory-isolated *Acinetobacter schindleri* ACE strain that quickly and efficiently catabolizes acetate

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Abstract

An *Acinetobacter* strain, designated ACE, was isolated in the laboratory. Phylogenetic tests and average nucleotide identity value comparisons suggested that ACE belongs to the species *Acinetobacter schindleri*. We report for the first time the complete genome sequence of an *A. schindleri* strain, which consists of a single circular chromosome of 3 001 209 bp with an overall DNA G+C content of 42.9 mol% and six plasmids that account for 266 844 bp of extrachromosomal material. The presence or absence of genes related to carbon catabolism and antibiotic resistance were in agreement with the phenotypic characterization of ACE. This strain grew faster and with a higher biomass yield on acetate than the reference strain *Acinetobacter baylyi* ADP1. However, ACE did not use aromatic compounds and was unable to grow on common carbon sources, such as glucose, xylose, glycerol or citrate. The gluconeogenic and the catechol pathways are complete in ACE, but compounds that are converted to protocatechuate did not sustain growth since some genes of this pathway are missing. Likewise, this strain could not grow on glucose because it lacks the genes of the Entner–Doudoroff pathway. Minimal inhibitory concentration data showed that ACE was susceptible to most of the antimicrobial agents recommended for the clinical treatment of *Acinetobacter* spp. Some genes related to a possible human–microbe interaction were found in the ACE genome. ACE is likely to have a low pathogenic risk, as is the case with other *A. schindleri* strains. These results provide a valuable reference for broadening the knowledge of the biology of *Acinetobacter*.

INTRODUCTION

The genus *Acinetobacter* belongs to the order *Pseudomonadales* and comprises strictly aerobic, non-fermentative, Gram-negative bacteria found in different habitats, including water, soil and even human skin. These bacteria are ubiquitous and considered free-living saprophytes [1]. Some species, such as *Acinetobacter baumannii*, can be pathogenic and are considered opportunistic with a special incidence in nosocomial environments [2]. By contrast, the non-pathogenic *Acinetobacter baylyi* strain ADP1 is of notable biotechnological interest because of its remarkable natural transformation and genetic recombination capabilities [3]

and its ability to degrade aromatic compounds [4]. This strain has been proposed as a model organism owing to its metabolic versatility, fast growth rate, easy cultivation, genome sequence availability and ease of genetic manipulation [5, 6]. Such diversity of habitat and metabolic capabilities highlights the physiological and genetic flexibility of members of this genus, which enables them to survive under highly diverse conditions that can sometimes be associated with pathogenicity. However, despite the extensive research on *A. baumannii* and *A. baylyi* ADP1, only a few reports of the complete genomes of other *Acinetobacter* species exist that can explain the basis of the adaptation to different environments. That is the case for *Acinetobacter schindleri*,

Received 16 November 2016; Accepted 19 May 2017

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Keywords: *Acinetobacter*; genome sequence; acetate catabolism; microbial physiology.

Abbreviations: μ , specific growth rate; ANIm, average nucleotide identity; BLAST, basic local alignment search tool; CoA, coenzyme A; COG, cluster of orthologous groups; DCW, dry cell weights; MIC, minimal inhibitory concentration; PHB, p-hydroxybenzoate; *q*_{Ac}, specific acetate consumption rate; $Y_{x/s}$, biomass yield on substrate.

The GenBank/EMBL/DDBJ accession numbers for the chromosome and plasmid sequences of *A. schindleri* ACE are CP015615 (AsACE for chromosome), CP015616 (p1AsACE for plasmid 1), CP015617 (p2AsACE for plasmid 2), CP015618 (p3AsACE for plasmid 3), CP015619 (p4AsACE for plasmid 4), CP015620 (p5AsACE for plasmid 5) and CP015621 (p6AsACE for plasmid 6).

which has been isolated from clinical samples [7, 8] and activated sludge [9].

In nature, micro-organisms are frequently exposed to dynamic changes in the availability of carbon sources and must, therefore, adapt quickly to these changing environmental conditions. A good example of this situation is when certain micro-organisms first deplete acetogenic carbon sources from the environment and start to use acetate as a substrate, which is mainly considered a by-product [10, 11]. The assimilation of acetate requires gluconeogenic activities and has been explored in many micro-organisms and studied extensively in *Escherichia coli* [11–14].

Here, we report on the physiological characterization of an *A. schindleri* strain designated ACE that was isolated from the laboratory as a contaminant. This strain is of great interest because it grows quickly and to a high cell mass using acetate as its sole carbon source. However, it is unable to catabolize other common carbon sources, such as glucose or phenolic compounds. To better understand the genetic basis of these peculiar metabolic features, the complete genome sequence of this strain was obtained and analysed to correlate the phenotypic characteristics observed under laboratory conditions with the metabolic pathways predicted from the genome. We also attempted to identify factors that would contribute to the knowledge of the possible interactions of this strain with humans.

METHODS

Isolation and identification of *A. schindleri* ACE

The bacterium under study was isolated from the laboratory environment as a contaminant of an *E. coli* mutant strain PB12 *maeB*::lx cultivated on M9 solid mineral media (see below) supplemented with acetate (3 g l^{-1}) and kanamycin ($30 \text{ } \mu\text{g ml}^{-1}$) [14]. Single colonies were obtained by streaking on Luria–Bertani (LB) plates with kanamycin ($30 \text{ } \mu\text{g ml}^{-1}$) and used to inoculate M9 liquid mineral medium supplemented with $3 \text{ g sodium acetate l}^{-1}$ and $30 \text{ } \mu\text{g kanamycin ml}^{-1}$. Once in the exponential phase, samples were collected from the liquid culture and streaked on LB plates with kanamycin ($30 \text{ } \mu\text{g ml}^{-1}$) to obtain single colonies. These single colonies were then used to start new cultures in M9 liquid mineral medium containing both acetate and kanamycin. The average specific growth rate (μ) of the isolates retrieved in this medium was approximately 0.9 h^{-1} . This value was used as a parameter to distinguish the colonies isolated from the *E. coli* mutant PB12 *maeB*::lx that has a μ of 0.15 h^{-1} under the same growth conditions with acetate as the sole carbon source [14]. This procedure was repeated several times to purify the strain, and the isolate obtained was ultimately named ACE. Genomic DNA was extracted, and the 16S rRNA gene was amplified by PCR (primers used: 5' AGAGTTTGATCCTGGCTCAG 3' and 5' GGTTACCTTGTTACGACTT 3'), cloned into a suitable vector (pGEM-T, Promega) and sequenced [15]. The 16S rRNA gene sequence obtained was compared with those in the GenBank (NCBI) databases using the basic local

alignment search tool (BLAST) algorithm [16]. For the phylogenomic tree, all the genes of 14 *Acetivibrio* strains were clustered using the MCL software [17], and the common core of genes was obtained. The predicted protein sequences of these common genes, corresponding to 278 780 amino acids without gaps, were then joined and aligned with CLUSTAL X [18]; the RAXML software was used to build the tree using the PROTGAMEWAG method with 1000 bootstrapping replications [19].

Genome sequencing, assembly and annotation

Genomic DNA from ACE was sheared to produce paired-end libraries of 300 bp inserts or mate-paired libraries, one with 3 kb inserts and the other with 8 kb inserts, and sequenced using an Illumina MiSeq sequencer with a 2×250 cycle run. The total number of reads was 13 367 718 paired reads with a genome coverage of $1000 \times$. Library construction and sequencing was performed at Mogene LC (St. Louis, MO, USA). Different assembly strategies were used with the following programs: Velvet 1.1.06 [20], Sspace-Basic 2.0 [21] and Consed v23 [22]. ORFs were predicted using Glimmer 3.02 [23], and annotations were performed using Artemis 12.0 [24], a graphic display comparing the non-redundant databases of GenBank [25], Conserved Domain of GenBank [26], Interpro [27] and ISfinder [28].

Sequence analysis

Average nucleotide identity (ANI_m) with MUMmer was calculated using the JSpecies software [29]. The DNA conservation between two genomes or replicons was estimated by obtaining an alignment with NUCmer [30] using default parameters. The summed lengths of all aligned regions were then divided by the length of the genome or replicon, and all values were expressed as a percentage. Common and specific protein families between different *Acinetobacter* species were defined as clusters of the predicted protein products of genes showing a minimum 40 % identity and 80 % coverage using MCL [17]. The metabolic pathways of ACE were obtained by matching the predicted proteins to the KEGG pathway database using BLASTP [31].

Growth media and cultivation conditions

M9 mineral medium was prepared as follows: $6 \text{ g Na}_2\text{HPO}_4 \text{ l}^{-1}$, $0.5 \text{ g NaCl l}^{-1}$, $3 \text{ g KH}_2\text{HPO}_4 \text{ l}^{-1}$, $1 \text{ g NH}_4\text{Cl l}^{-1}$, $240.9 \text{ mg MgSO}_4 \text{ ml}^{-1}$, $11.1 \text{ mg CaCl}_2 \text{ l}^{-1}$, $0.02 \text{ mg FeSO}_4 \cdot 7\text{H}_2\text{O ml}^{-1}$ and $2.0 \text{ mg vitamin B1 l}^{-1}$. Frozen vials stored at -80°C with glycerol (25 %, v/v) were used to start the inocula on LB broth ($5 \text{ g yeast extract l}^{-1}$, $10 \text{ g tryptone l}^{-1}$, 10 g NaCl l^{-1}) plates (1.5 % agar, w/v), which were incubated overnight at 30°C . Isolated colonies were then cultivated in 250 ml shake flasks with 50 ml M9 liquid medium containing $3 \text{ g sodium acetate l}^{-1}$ for 12 to 18 h at 30°C and an agitation speed of 250 r.p.m. ACE cultures were supplemented with $30 \text{ } \mu\text{g kanamycin ml}^{-1}$. Bioreactor cultivations were performed in a 1 l BiostatB Plus bioreactor (Sartorius) containing 0.60 l M9 medium supplemented with $5.5 \text{ g sodium acetate l}^{-1}$ and inoculated at an initial optical density of 0.1 at 600 nm ($\text{OD}_{600 \text{ nm}}$). The reactor conditions were as follows: 30°C ,

constant aeration rate of 1 v.v.m. (volume of air per volume of medium per min), pH 7 maintained by automatic addition of 10 % (v/v) HCl and dissolved oxygen tension >20 % with respect to air saturation as maintained by increasing the stirrer speed (initial stirred speed was 200 r.p.m.). Cultivations in M9 liquid medium with 2 g additional single carbon source l^{-1} , such as glucose, xylose, arabinose, glycerol, succinate, citrate, *p*-hydroxybenzoate (PHB), vanillate, lactate, malate or ethanol, were performed in 250 ml shake flasks at 30 °C and 250 r.p.m.

Analytical methods

Bacterial growth was monitored spectrophotometrically at 600 nm (Genesys 20, Thermo Scientific). For bioreactor cultivations, biomass formation was determined gravimetrically. Dry cell weights (DCWs) were obtained from cell pellets dried at 80 °C for at least 18 h. A calibration curve was obtained to correlate absorbance measurements at 600 nm with DCW, and the following factor was obtained: $1 \text{ OD}_{600 \text{ nm}} = 0.55 \text{ g}_{\text{DCW}} l^{-1}$. Acetate levels were determined using a Varian HPLC system with an Aminex HPX-87H column according to the manufacturer's instructions (Bio-Rad).

Susceptibility test

Minimal inhibitory concentrations (MICs) were determined according to the guidelines published by the Clinical and Laboratory Standards Institute [32]; MicroScan panels NC32 and NC20 (Dade Behring) were used. MICs for kanamycin, streptomycin and spectinomycin were determined by agar microdilution according to the methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically [33].

RESULTS AND DISCUSSION

Discovery of the ACE strain

In the course of experimental study of an *E. coli* mutant, strain PB12 *maeB::lx* [14], we recorded a strain showing rapid growth on minimal medium with acetate as the sole carbon source. After several purification steps by clonal propagation of the strain starting with unique colonies, and testing for the phenotype of the original *E. coli* PB12 *maeB::lx*, it was clear that a bacterium different from the initial one was growing in the acetate cultures with higher μ (0.9 h^{-1}). This was corroborated by PCR of the 16S rRNA gene and by comparing the sequence of the conserved fragment of 889 bp with sequences in the Genbank database. We found that this strain belongs to the genus *Acinetobacter* and that it is very closely related to *A. schindleri* LUH5832 with a sequence identity of 98 %. This strain was Gram-negative and resistant to kanamycin and was denominated ACE as an acronym of its property of growth in acetate.

The reduced genome of the ACE strain

The complete genome sequence of ACE was obtained as a part of its initial characterization. It has a single circular chromosome of 3 001 209 bp and an overall DNA G+C content of 43 mol%. Indeed, the ACE strain harbours six plasmids of different length and lower DNA G+C content (about 34 to

40 mol%) than the chromosome. Several *Acinetobacter* strains harbour plasmids, and ACE is one of the strains with the largest amount of this genetic material [34] accounting for 260 kb of the total genome length. As a whole, the ACE genome contains 3021 genes, 37 pseudogenes, 21 rRNA and 84 tRNA.

Genomic comparisons of the ACE strain with respect to other *Acinetobacter* genome sequences confirmed that this strain belongs to the species *A. schindleri* (Fig. 1). The ANIm values were around 97 % for the species *A. schindleri* and below 88 % for other *Acinetobacter* species. According to these results, the ACE strain maintains a clear chromosomal synteny with respect to the scaffolds of *A. schindleri* NIPH900 and CIP 107287 (Fig. 2a, b). In contrast, the overall genome synteny of ACE with respect to two of the most representative strains of the genus, the pathogenic *A. baumannii* AYE and the non-pathogenic *A. baylyi* ADP1, gives an 'X-like' pattern, which suggests that these chromosomes have undergone multiple inversions along the species separation (Fig. 2c, d). A phylogenomic tree reconstructed using the maximum-likelihood method and linking all common gene-coding protein sequences of the strains analysed clustered the ACE strain with *A. schindleri* (Fig. 2e). This tree also shows that the monophyletic group comprised of the *A. schindleri* and *Acinetobacter lwoffii* species is clearly different with respect to the group formed by the other *Acinetobacter* species, including *A. baumannii*. *A. schindleri* was described in 2001 [7] and is considered to have a low pathogenic potential, being mainly opportunistic in immunocompromised patients who are hospitalized for long periods of time [8]. Although most *Acinetobacter* species have been isolated from clinical sources, many other species have been retrieved from environmental sources [9].

Most of the *Acinetobacter* genomes reported thus far have larger genomes (approximately 3.5–4 Mb) in comparison with the relatively small chromosome of ACE [35]. This suggests that this strain has undergone a process of chromosome reduction. Comparing the predicted protein content of *A. schindleri* ACE with those of the pathogenic strain *A. baumannii* AYE and the non-pathogenic strain *A. baylyi* ADP1, 1818 protein clusters with 5809 proteins were found that are shared among the three genomes (Fig. 3a). Interestingly, ACE has fewer specific proteins (790), while AYE and ADP1 share more proteins among themselves (916) than each does with ACE. When comparing the complete clusters of orthologous groups (COGs) of proteins between *A. schindleri* ACE, *A. baumannii* AYE and *A. baylyi* ADP1 (Fig. 3b), ACE has fewer genes mainly in the COGs related to transport and metabolism of amino acids, lipids, carbohydrates and secondary metabolites (E, I, G and Q, respectively). Fewer genes in these functions could explain, in part, ACE's limited use of common carbon sources (Table S1, available in the online Supplementary Material). Finally, ACE has acquired exogenous DNA within its chromosome or plasmids, as these are functions that prevail in the extra-chromosomal elements.

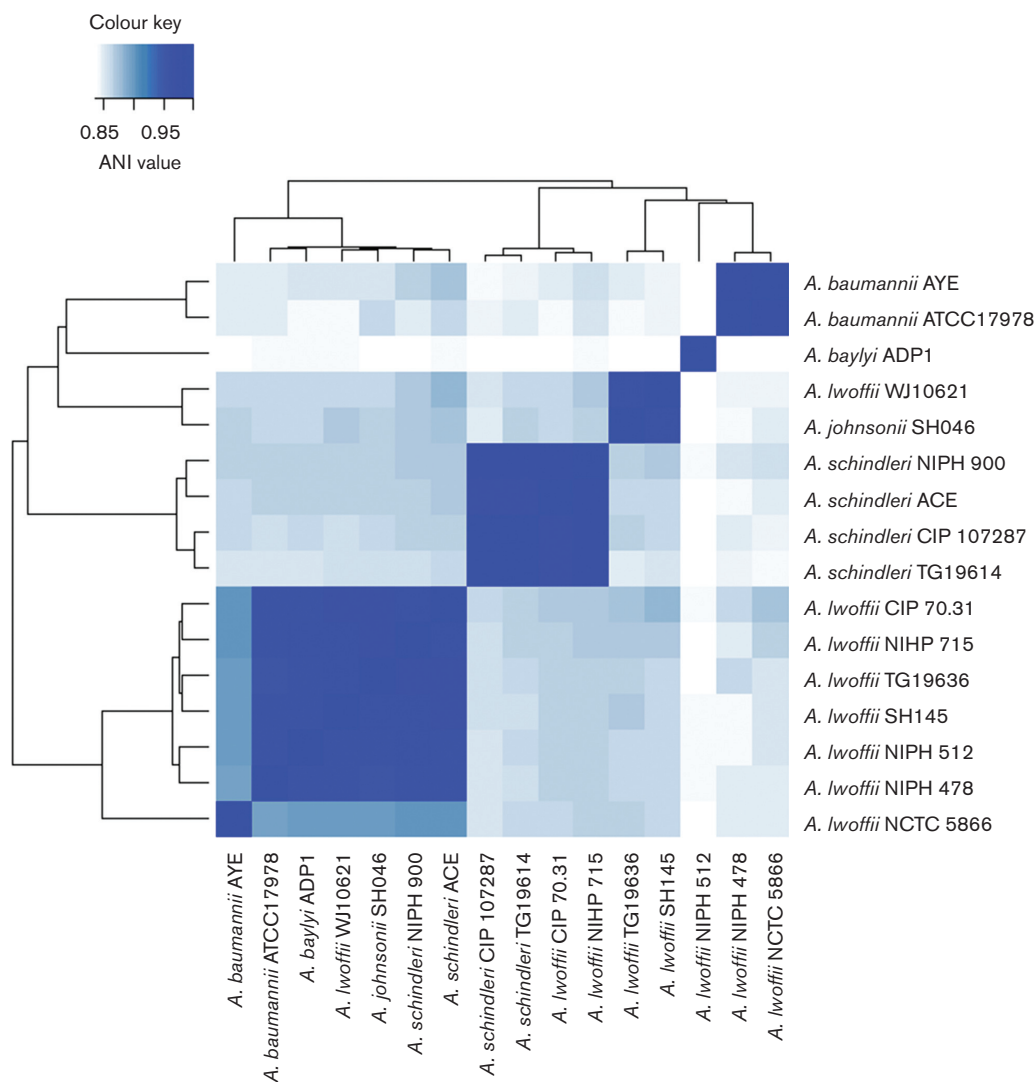


Fig. 1. Representation of the average nucleotide identity based on MUMmer (ANIm) among different sequences of *Acinetobacter* spp. using the JSpecies software. Ramifications show clusters formed according with the percentage of similarity between sequences.

ACE's limited use of common carbon sources

Of the substrates used in this work (Table 1), ACE grew well only on acetate and lactate, with limited growth on succinate and ethanol. The ability to grow on acetate and lactate is common in different *A. schindleri* strains, while malate and ethanol are utilized only by certain strains [7]. The utilization of succinate and lactate as carbon sources has been reported elsewhere for strains of different *Acinetobacter* species, including *A. baylyi* ADP1 [36–38]. However, while ADP1 could catabolize citrate, ACE was unable to use this organic acid as a carbon source. Moreover, ACE was unable to grow on common carbon sources such as glucose, xylose, arabinose and glycerol (Table 1). In contrast, ADP1 grew on glucose and glycerol but was unable to use xylose or arabinose as carbon sources.

The breakdown of aromatic compounds has been studied extensively in *Acinetobacter* species. In these species,

aromatic compounds are first degraded to protocatechuate or catechol before being funnelled to the β -ketoacid pathway and eventually incorporated into the central carbon metabolism pathway via acetyl coenzyme A (CoA) and succinyl-CoA [36, 39]. This pathway is well characterized in *A. baylyi* ADP1 [40], and thus we tested whether the ACE strain was also able to use aromatics. Two compounds, PHB and vanillate, which are converted to protocatechuate and are used by ADP1 as carbon sources, did not sustain ACE growth (Table 1), although it is reported that most of the *A. schindleri* strains are able to catabolize PHB [7].

ACE clearly has a sort of specialization to use acetate as a carbon source. It is possible that this strain evolved to catabolize compounds that ultimately are metabolized gluconeogenically via acetyl-CoA, like dicarboxylic acids and

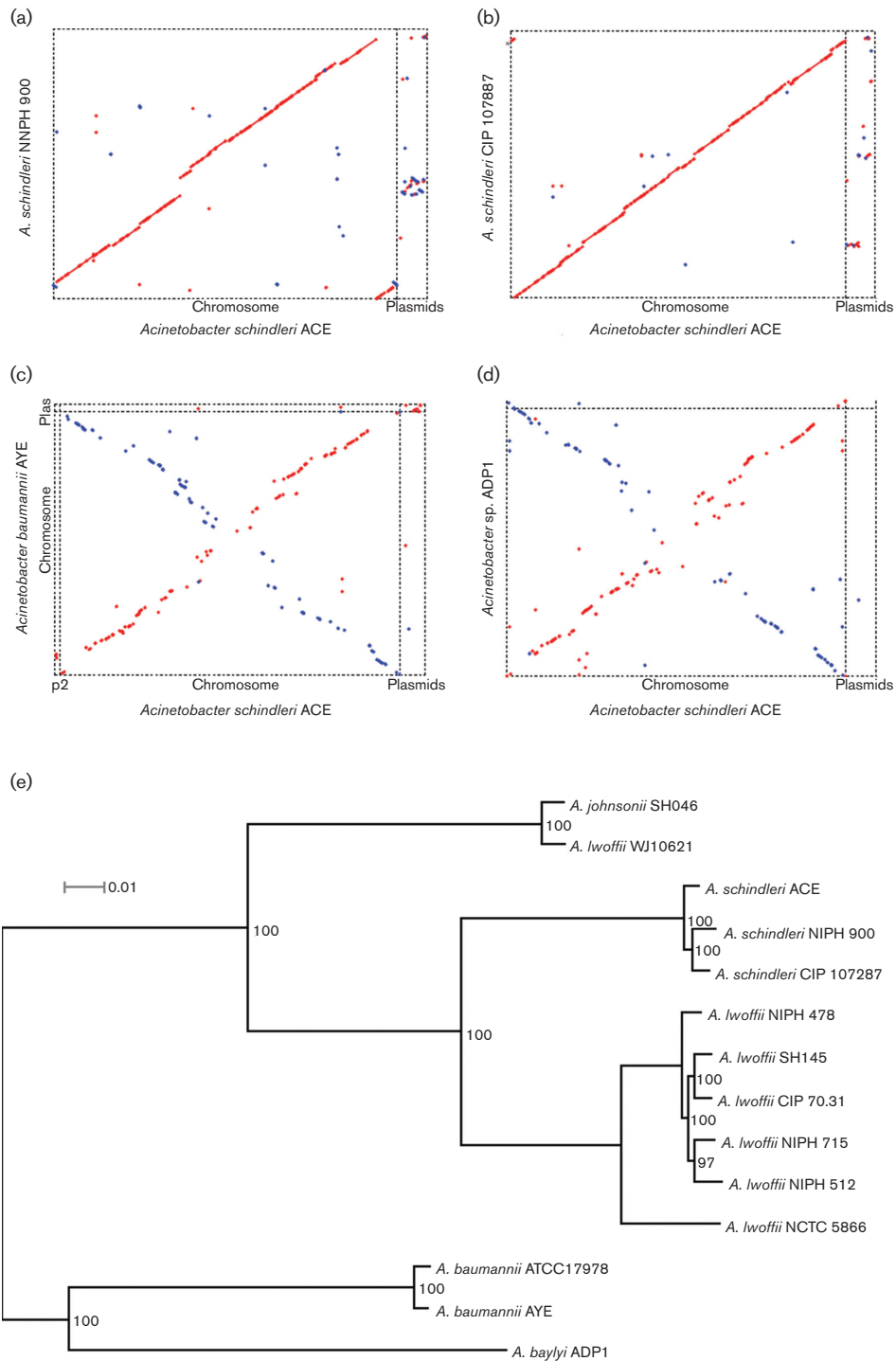


Fig. 2. Synteny plots of the complete genome sequences of *A. schindleri* ACE (x-axis) in comparison with *A. schindleri* NIPH900 (a), *A. schindleri* CIP 107287 (b), *A. baumannii* AYE (c) and *A. baylyi* ADP1 (d), and phylogenomic relationships of *Acinetobacter* species (e). Bootstrap values are indicated at the nodes of the tree. Bar, 1 change for every 100 amino acids.

fatty acids. This phenotypic peculiarity could be associated with the possible participation of ACE as part of the human skin microbiome. Some micro-organisms, like *Propionibacterium acnes*, hydrolyse triglycerides present in sebum and

release free fatty acids onto the skin [41]. These compounds excreted as carbon wastes could be used as substrates by ACE, which has genes coding for fatty acid transport and degradation (Fig. S1).

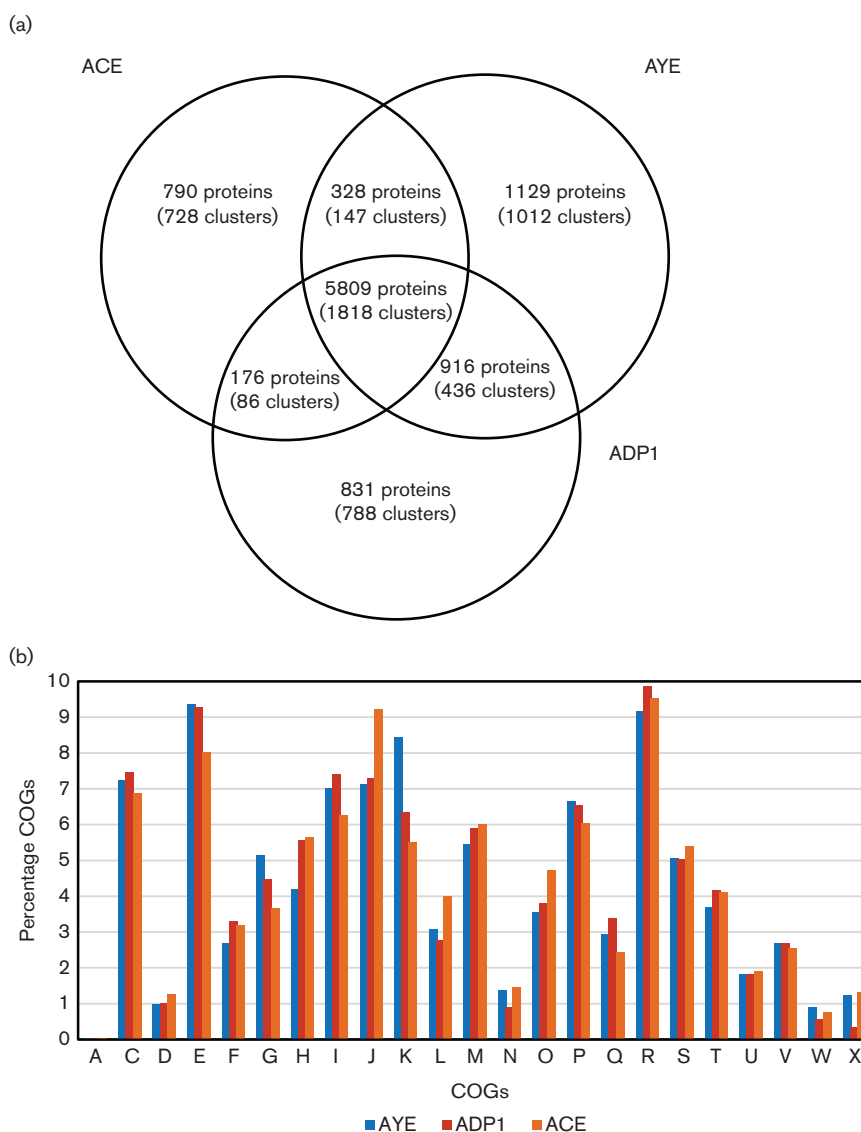


Fig. 3. (a) Predicted protein content and protein clusters comparison of *A. schindleri* ACE, *A. baumannii* AYE and *A. baylyi* ADP1. The orthologues are defined as predicted protein products of genes showing a minimum of 40% identity and 80% coverage. The intersections between the circles are the number of proteins or protein clusters found in two or three micro-organisms, while proteins outside the intersections are specific to each strain. (b) Percentages of clusters of orthologous groups (COGs) of proteins of the *A. schindleri* ACE complete genome and comparison with the COGs of the *A. baumannii* AYE and *A. baylyi* ADP1 genomes. COG classes: (A) RNA processing and modification; (C) energy production and conversion; (D) cell cycle control, cell division, chromosome partitioning; (E) amino acid transport and metabolism; (F) nucleotide transport and metabolism; (G) carbohydrate transport and metabolism; (H) coenzyme transport and metabolism; (I) lipid transport and metabolism; (J) translation, ribosomal structure and biogenesis; (K) transcription; (L) replication, recombination and repair; (M) cell wall/membrane/envelope biogenesis; (N) cell motility; (O) post-translational modification, protein turnover, chaperones; (P) inorganic ion transport and metabolism; (Q) secondary metabolites biosynthesis, transport and catabolism; (R) general function prediction only; (S) function unknown; (T) signal transduction mechanisms; (U) intracellular trafficking, secretion and vesicular transport; (V) defence mechanisms; (W) extracellular structures.

On the basis of these findings, we named our isolate *A. schindleri* ACE because of its growth limitations using common carbon sources in the laboratory, except for acetate.

Predicted catabolic pathways in ACE

Analysis of the metabolic pathways predicted by KEGG comparisons showed that the gluconeogenic pathway is

complete in the ACE strain (Fig. 4a), which is consistent with its growth on acetate or succinate. However, ACE could not utilize citrate, unlike ADP1 (Table 1), because it lacks the *tcuRABC* genes. *tcuR* encodes a regulatory protein required for the expression of the *tcuABC* operon in *Salmonella enterica*, which allows growth on tricarballoylate or related compounds such as citrate, *cis*-aconitate or isocitrate

Table 1. Growth of strains ACE and ADP1 in shake flasks containing liquid M9 medium with different carbon sources (2 g l⁻¹ each)

+, Growth; +/-, slight growth; -, no growth. Results determined after 24 h of incubation at 30°C and 250 r.p.m. These data come from at least two independent cultures, each test performed twice. Both strains grew on acetate and lactate, both showed slight growth on ethanol, and both showed no growth on xylose or arabinose.

Compound	<i>Acinetobacter</i> strain	
	ACE	ADP1
Glucose	-	+
Glycerol	-	+
Succinate	+/-	+
Citrate	-	+
PHB	-	+
Vanillate	-	+
Malate	-	+

[42]. TcuC is a transporter of tricarballylates, which are oxidized to aconitate by TcuB in concert with the flavoprotein TcuA. Highly homologous genes for these proteins were found in synteny in ADP1 [43].

With respect to glucose catabolism, *A. schindleri* ACE and *A. baylyi* ADP1, in addition to many other *Acinetobacter* species, lack important glycolytic enzymes, such as glucokinase, 6-phosphofructokinase and pyruvate kinase, as well as proteins of the phosphotransferase system [44]. Additionally, ACE lacks crucial genes such as *gntK* (a gluconate kinase), the genes *edd* and *eda* of the Entner–Doudoroff pathway, and the genes *ACIAD2984* and *gntT* that code for a putative glucose-sensitive porin and a high-affinity gluconate permease, respectively. Therefore, ACE is completely unable to utilize glucose (Table 1), contrary to other *Acinetobacter* strains, such as ADP1 that uses the Entner–Doudoroff pathway to catabolize this hexose.

By contrast, ADP1 utilizes glycerol as its sole source of carbon while ACE does not (Table 1). ADP1 contains the genes of the main pathway of glycerol utilization previously described in *E. coli* [45], which include a kinase (*glpK*) and a dehydrogenase (*glpD*). *glpK* is present on the ACE chromosome, but *glpD* is not. However, both ADP1 and ACE contain glycerol dehydrogenase encoded by *gpsA*. The main difference among these dehydrogenases is that GlpD uses quinones as cofactors, while GpsA requires NAD⁺(P). In *E. coli* there is also a glycerol uptake facilitator protein (GlpF) that has not been identified in ADP1 or ACE. It is possible that the ACE strain requires GlpD activity and/or a specific transporter not yet identified. As shown in Table 1, ADP1 and ACE were not able to use xylose or arabinose as carbon sources. Genome sequence analysis of ACE and comparison with the sequenced ADP1 genome [44] revealed the absence of essential genes (including *xylAB* and *araABD*) for the catabolism of these pentoses.

With regards to the catabolism of aromatic compounds, the catechol branch and β -ketoadipate pathway are complete in

ACE, but the protocatechuate branch is incomplete owing to the absence of *pcaBHG* genes (Fig. 4b). PcaHG are protocatechuate oxygenases that produce β -carboxymuconate, which is transformed to γ -carboxymuconolactone by the cycloisomerase PcaB. This carboxymuconolactone is catabolized in the ketoadipate pathway. Moreover, the mono-oxygenases encoded by *pobA* and *vanAB*, and required for *p*-hydroxybenzoate and vanillate utilization, respectively, are also absent in ACE. Genes associated with the catabolism of aromatic compounds are clustered on the chromosome of *A. baylyi* ADP1 in regions referred to as catabolic islands [4]. In ACE, some clusters of catabolic genes or genes within these clusters are missing, including *vanABRK* (cluster I, catabolism of vanillate to protocatechuate), *salARE-areABCR* (cluster II), *atsBCR* (cluster III) and *pcaUFBDKHG-aroD-quiC-pobRA-hcaGECA-mdcABC-DEGLMR* (cluster IV) (Fig. S2).

Human–microbe interaction-related genes found in the ACE genome

As a first step to determining the possible pathogenic capabilities of ACE, several virulence factors that have been described for *A. baumannii*, including drug resistance, iron metabolism, adhesion, motility, quorum sensing and two-component systems, were searched for in the ACE genome by means of genomic analysis [46–48] (Table 2). *Acinetobacter* species can grow in the presence of different antimicrobial agents [49]. To expand the phenotypic characterization of ACE, MIC analyses of common clinical antibiotics were performed (Table 3). Kanamycin was the antibiotic in which ACE isolation was conducted, and the MIC value for kanamycin indicated that this strain is able to grow at least up to 64 $\mu\text{g ml}^{-1}$. For other aminoglycosides, ACE showed clinical susceptibility to gentamicin, amikacin and tobramycin, and apparently intermediate resistance to streptomycin, as judged from the MIC values shown in Table 3. We identified two genes in the ACE genome (Table 2) that encode for aminoglycoside phosphotransferase proteins (AsACE_CH01525 and AsACE_CH01732) and two genes in plasmids 2 and 3 (AsACE_p200011, AsACE_p300003) that could potentially confer resistance to kanamycin and to some level to streptomycin [50, 51].

Of the compounds tested, ACE was susceptible to 12 antimicrobial agents and intermediately susceptible to meropenem, according to the MIC interpretive standards of the CLSI for *Acinetobacter* spp. (2015). Except for kanamycin, cefazoline and cefotetan, the MICs of antimicrobial agents for which there are no interpretative criteria for *Acinetobacter* spp. were low considering the scale used for *Enterobacteriaceae*, *Pseudomonas aeruginosa* or *Neisseria gonorrhoeae*. Contrary to the resistant *A. schindleri* MRSN 10319 [52], ACE was susceptible to ampicillin/sulbactam, cefepime and ceftazidime and had intermediate susceptibility to meropenem (Table 3). Although ACE has four genes encoding metallo- β -lactamases (AsACE_CH00142, AsACE_CH00315, AsACE_CH00933 and AsACE_CH02163), none of these appeared to have significant similarity to the

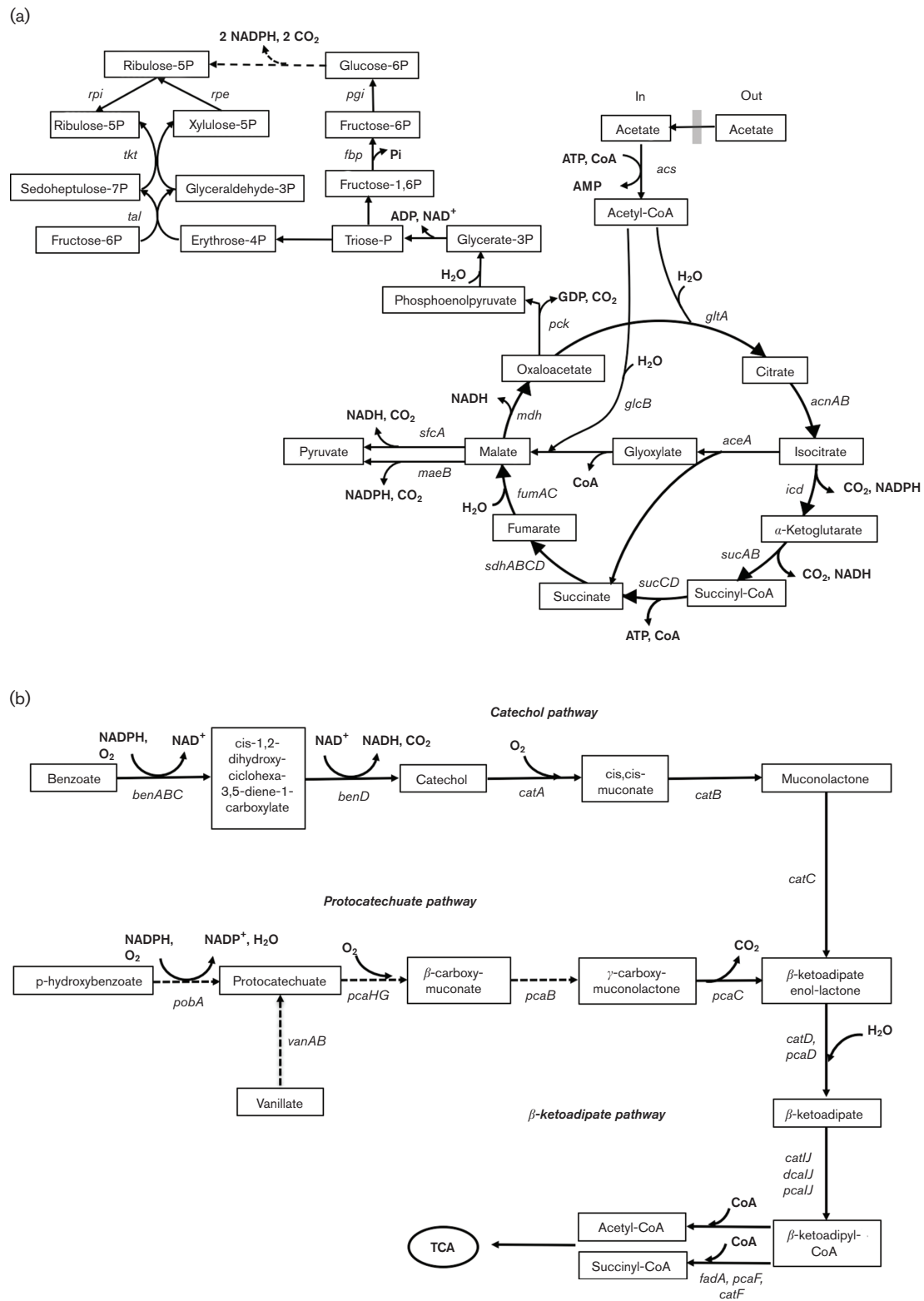


Fig. 4. Catabolism of acetate (a) and aromatic compounds (b) in *A. schindleri* ACE showing key metabolites and genes. Gene symbols are as follows: acetyl-CoA synthetase (*acs*), citrate synthase (*gltA*), aconitate hydratase (*acnAB*), isocitrate lyase (*aceA*), malate synthase (*glcB*), isocitrate dehydrogenase (*icd*), α -ketoglutarate dehydrogenase (*sucAB*), succinyl-CoA synthetase (*sucCD*), succinate dehydrogenase (*sdhABCD*), fumarate hydratase (*fumAC*), malate dehydrogenase (*mdh*), malic enzyme 1 (*sfcA*), malic enzyme 2 (*maeB*), phosphoenolpyruvate carboxykinase (*pck*), fructose-1,6-bisphosphatase (*fbp*), transaldolase (*tal*), transketolase (*tkt*), ribulose-phosphate 3-epimerase (*rpe*), ribose 5-phosphate isomerase (*rpi*). Absence of an associated gene abbreviation implies two or more

enzymic steps. Arrows show the main direction of the reaction. Dashed lines indicate the absence of the corresponding reaction in the ACE strain. Some relevant enzyme names for the catabolism of aromatic compounds are mentioned in the text. P, phosphate; TCA cycle, tricarboxylic acid cycle.

blaNDM-1 gene present in *A. schindleri* MRSN 10319 (data not shown). Interestingly, a gene for a carbapenemase (β -lactamase class D, *blaOxa-278*, AsACE_CH00016) is conserved among strains of *A. schindleri* and *A. lwoffii* species including the ACE strain, but it is not present in *A. baumannii* strains or in ADP1. Class D β -lactamase genes are frequent in many Gram-negative rods but it is known that they play a minor role in resistance phenotypes of *Acinetobacter* spp. thriving in non-clinical environments [53]. However, overproduction of class D β -lactamase mediated by several factors associated with the clinical environment could contribute to expansion of the resistance phenotypes to different β -lactams, including carbapenems. In this context, ACE shows intermediate resistance to meropenem (Table 3), and although other factors, including low outer membrane permeability and efflux pumps may be involved, it is possible that OXA-278 overproduction also contributes to this resistance.

The use of a β -lactamase inhibitor, like clavulanate, in combination with cefotaxime or ceftazidime reduced the MIC significantly (Table 3). Although the effect of clavulanate alone was not tested, this most likely reflects alterations in penicillin-binding proteins by clavulanic acid rather than inhibition of an extended-spectrum β -lactamase as shown for another *Acinetobacter* species [54].

Microbes that interact with humans require efficient iron uptake systems to survive during iron scarcity and to successfully grow and invade hosts. There is a wide distribution of multiple iron-acquisition systems among clinical isolates of *A. baumannii* [55, 56]. Likewise, there are two ferrous [Fe(II)] iron uptake systems (FeoAB) in ACE (Table 2), encoded by genes on the chromosome (AsACE_CH02667-*feoB* and AsACE_CH02668-*feoA*) and on plasmid 1 (AsA122_p100053-*feoA*). These genes are normally arranged in operons along with *feoC*, which encodes a transcriptional regulator [56]. The integral large membrane FeoB protein functions as the Fe²⁺ permease, while the cytosolic small FeoA protein is thought to be required for maximal FeoB activity [57]. It is well known that bacterial cells can also uptake Fe(III)-loaded siderophores by specific receptor proteins in an energy-dependent mechanism that mainly involves the protein complex TonB/ExbB/ExbD [58]. On the ACE chromosome, we identified the genes *tonB* (AsACE_CH00410 and AsACE_CH02443), *exbB* (AsACE_CH00411, AsACE_CH01727 and AsACE_CH01944) and *exbD* (AsACE_CH00412, AsACE_CH001726 and AsACE_CH01945). None of the genes involved in acinetobactin synthesis and transport were found in the ACE genome (Table 2) [59]. By contrast, to avoid toxic intracellular iron concentrations, a transcriptional repressor of genes involved in iron homeostasis (Fur) has been well characterized in *E. coli* [60]. ACE has two *fur* genes in its chromosome (AsACE_CH00694 and AsACE_CH02742), which suggested

complex regulation of iron metabolism in this bacterium. In fact, iron is the only trace element necessary for growth of ACE in minimal medium (see Methods).

Some other factors that could be implicated in human-microbe interaction in ACE are OmpA, phospholipase D and extracellular polysaccharide-associated proteins. Two genes were found in the ACE chromosome that code for OmpA, AsACE_CH02243 and AsACE_CH01053, but only the former of these products has a percentage of identity greater than 80% and is similar in length compared with a representative *A. baumannii* sequence (Table 2). OmpA is the most abundant outer membrane protein and it has been associated with multiple functions in the pathogenesis of *A. baumannii*, which include biofilm formation, adherence and induction of apoptosis [61, 62]. ACE has three genes that code for phospholipase D (AsACE_CH00557, AsACE_CH02260 and AsACE_CH01516) but none for phospholipase C (Table 2). The proposed role of phospholipase D is serum resistance and bacterial dissemination [63]. Finally, we identified in ACE the locus *pgaABCD* (AsACE_CH00825, AsACE_CH00826, AsACE_CH00827 and AsACE_CH00828), whose products are associated with the extracellular polysaccharide poly- β -1,6-*N*-acetyl-D-glucosamine that is important in the biofilm formation of *A. baumannii* [48, 64]. However, these proteins had a low percentage of identity compared with *A. baumannii* sequences (Table 2).

Some of the pathogenic determinants described for *A. baumannii* that are absent in the ACE genome include CsuA/BABCDE chaperone-usher pili assembly system, biofilm-associated protein (Bap), AbaI autoinducer synthase, two-component regulatory system (BfmRS), penicillin-binding protein 7/8 (PBP-7/8) and lipopolysaccharide biosynthesis proteins (LpsB), among others [47, 48, 62, 65].

It is well known that there is no single factor associated with virulence in *Acinetobacter* [47, 62, 66]. In fact, the pathogenicity of *A. baumannii* is a multifactorial and combinatorial phenomenon that is associated with the presence of many determinants and their specific regulation [67]. As mentioned, *A. schindleri* strains are considered to have low pathogenic potential [8] and it is important to note that even non-pathogenic *Acinetobacter* strains can express virulence-associated elements [47]. Thus, it is relevant to point out that some of the genes described in this section could be necessary for *A. schindleri* strains to interact with humans but not in the context of pathogenicity. However, further studies are required to assess the nature of this host-microbe interaction.

Fast growth of ACE using acetate as a sole carbon source

The main phenotypic characteristic displayed by ACE was its remarkably fast growth using acetate as its sole carbon source.

Table 2. Main pathogenic gene determinants described for *A. baumannii* and their presence or absence in the ACE genome

Description	Locus tag	Amino acids	Best blastp match with <i>A. baumannii</i> sequences		
			Identity (%)	Amino acids	Sequence ID
1 Antibiotic resistance					
Class D β -lactamase	AsACE_CH00016	276	97	276	WP_000854010.1
Aminoglycoside phosphotransferase	AsACE_CH01525	373	88	372	WP_034702769.1
	AsACE_CH01732	337	81	337	WP_032053788.1
	AsACE_p200011	271	99	271	WP_038349982.1
	AsACE_p300003	294	99	294	WP_064987035.1
2 Fe(II) iron uptake systems					
Ferrous iron transporter, FeoA	AsACE_CH02668	98	69	99	WP_000288742.1
	AsACE_p100053	81	97	80	WP_034706642.1
Ferrous iron transporter, FeoB	AsACE_CH02667	618	85	617	WP_032010068.1
FeoC	NF				
3 Fe(III)-loaded siderophores					
TonB family transporter	AsACE_CH00410	257	80	258	WP_034702349.1
	AsACE_CH02443	290	80	263	EXA65478.1
Transporter ExbB/TolQ 1	AsACE_CH00411	206	90	207	WP_034702346.1
	AsACE_CH01727	212	67	211	WP_032053791.1
	AsACE_CH01944	232	84	232	WP_000224628.1
Transporter ExbD/TolR 1	AsACE_CH00412	134	96	134	WP_034702344.1
	AsACE_CH01726	140	84	142	WP_000669682.1
	AsACE_CH01945	149	70	150	WP_001007564.1
Haem oxygenase (<i>hemO</i>)	AsACE_CH00901	470	66	473	WP_034703763.1
Acinetobactin synthesis and transport proteins	NF				
4 Outer membrane protein A (OmpA)	AsACE_CH02243	349	85	347	WP_000777884.1
5 Phospholipase D	AsACE_CH00557	487	85	487	WP_034704256.1
	AsACE_CH02260	544	81	544	WP_034704143.1
	AsACE_CH01516	518	69	517	WP_034704163.1
6 Phospholipase C	NF				
7 Capsule formation (<i>ptk</i> and <i>epsA</i>)	NF				
8 Extracellular polysaccharide					
Poly-N-acetylglucosamine (PNAG) export porin PgaA	AsACE_CH00825	817	44	800	WP_031979565.1
PNAG PgaB	AsACE_CH00826	601	45	608	WP_031948814.1
PNAG export synthase PgaC	AsACE_CH00827	420	73	415	WP_032038754.1
PNAG biosynthesis PgaD	AsACE_CH00828	193	48	154	WP_039247318.1
PNAG domain-containing protein	AsACE_CH00829	400	39	419	EXU12217.1

NF, not found.

ACE and *A. baylyi* ADP1 (=DSM 24193) as reference strain [3] were cultivated in bioreactors with 5.5 g acetate l⁻¹, in which a dissolved oxygen tension of >20 % (with respect to air saturation) and a constant pH of 7 were maintained. Under these conditions, ACE had a μ of 0.89 h⁻¹ and ADP1 had a μ of 0.44 h⁻¹ (Table 4). Additional cultivation parameters showed that the biomass yield of ACE on acetate ($Y_{x/s}$) was 2.4-fold higher than that of ADP1, while the specific acetate consumption rate (q_{Ace}) was 14 % lower in ACE compared with that in ADP1 (Table 4). These findings demonstrated a more efficient acetate catabolism from ACE than from ADP1. Different values of μ have been reported for *Acinetobacter* species using acetate as a substrate, i.e. for *Acinetobacter calcoaceticus*, 0.7 h⁻¹ [68], and for an *Acinetobacter* sp. HO1-N,

0.59 h⁻¹ [69]. In carbon-limited chemostat cultures, the μ_{max} for *A. calcoaceticus* using acetate was 1.22 h⁻¹ [70]. The results of these past studies, together with the values obtained here for the ACE and ADP1 strains, demonstrated that the *Acinetobacter* species generally grow quickly on acetate.

Concluding remarks

Here, we present the first complete assembly, annotation and analysis of a sequenced genome of an *A. schindleri* strain. The genomic characterization of ACE includes a novel comparison of its predicted protein content and protein clusters, and the percentage of COGs of proteins shared between *A. schindleri* ACE, the non-pathogenic *A. baylyi* ADP1 and the pathogenic *A. baumannii* AYE. Some genes

Table 3. Antimicrobial susceptibility profile of *A. schindleri* ACE strain

These data come from two independent cultures and MIC ($\mu\text{g ml}^{-1}$) determinations. MIC interpretive standards for *Acinetobacter* spp. are shown according to the Clinical and Laboratory Standards Institute [73] unless otherwise established.

Antibiotic	MIC of ACE	Interpretation	MIC range
Penicillins			
Ampicillin	<=8	NA	<=8, 16, >=32 ^{a*}
Ampicillin/sulbactam	<=8/4	S	<=8/4, 16/8, >=32/16
Ticarcillin/clavulanate	<=16	S	<=16/2, 32/2–64/2, >=128/2
Cephems			
Cefazoline	>16	NA	<=2, 4, >=8 ^a
Cefepime	3	S	<=8, 16, >=32
Cefotaxime	8	S	<=8, 16–32, >=64
Cefotaxime/clavulanate	<=0.5	NA	NA
Cefotetan	32	NA	<=16, 32, >=64 ^a
Ceftazidime	4	S	<=8, 16, >=32
Ceftazidime/clavulanate	<=0.25	NA	NA
Ceftriaxone	<=8	S	<=8, 16–32, >=64
Cefuroxime	6	NA	<=8, 16, >=32 ^a
Monobactams			
Aztreonam	<=8	NA	<=8, 16, >=32 ^b
Penems			
Meropenem	<=4	I	<=2, 4, >=8
Aminocyclitols			
Spectinomycin	16	NA	<=32, 64, >=128 ^c
Aminoglycosides			
Amikacin	<=4	S	<=16, 32, >=64
Gentamicin	<=1	S	<=4, 8, >=16
Kanamycin	>64	NA	<=16, 32, >=64 ^a
Streptomycin	64	NA	NA
Tobramycin	<=1	S	<=4, 8, >=16
Folate pathway inhibitors			
Trimethoprim/sulfamethoxazole	<=2/38	S	<=2/38, >=4/76
Fluoroquinolone			
Ciprofloxacin	<=1	S	<=1, 2, >=4
Levofloxacin	<=2	S	<=2, 4, >=8
Moxifloxacin	<=2	NA	NA

S, susceptible; I, intermediate; R, resistant; NA, not available.

*As reference, MIC interpretive standards according to the CLSI [73] for: a, *Enterobacteriaceae*; b, *Pseudomonas aeruginosa*; c, *Neisseria gonorrhoeae*.

related to a possible human–microbe interaction were found in the ACE genome, such as genes for iron metabolism, outer membrane protein A and phospholipase D, but some other virulence determinants of *A. baumannii* are lacking in ACE. It is possible that, similar to other *A. schindleri* strains reported, ACE has a low pathogenic risk. The presence or

Table 4. Specific rates of growth (μ) and acetate consumption (q_{Ace}) calculated during exponential growth, and cell mass yield on consumed acetate ($Y_{X/S}$) calculated at the depletion of acetate

Experiments were performed in bioreactors and using 5.5 g acetate l^{-1} . \pm , Standard error of duplicate experiments.

Parameter (units)	<i>Acinetobacter</i> strain	
	ACE	ADP1
μ (h^{-1})	0.89 \pm 0.06	0.44 \pm 0.01
$Y_{X/S}$ ($\text{gDCW g}_{\text{Ace}}^{-1}$)	0.43 \pm 0.06	0.18 \pm 0.01
q_{Ace} ($\text{g}_{\text{Ace}} \text{g}_{\text{DCW}}^{-1} \text{h}^{-1}$)	2.06 \pm 0.10	2.39 \pm 0.07

absence of genes related to carbon catabolism or antibiotic resistance is in agreement with the phenotypic characterization of this strain. Moreover, the catechol pathway is complete in ACE, but compounds that are converted to protocatechuate did not sustain growth since some genes of this pathway are missing. It is worth noting that *A. schindleri* ACE can catabolize only gluconeogenic substrates such as acetate or succinate, but it is unable to utilize aromatic compounds or hexoses as carbon sources. In fact, the most notable phenotypic property of ACE is its fast growth and high biomass yield on acetate compared with the model strain *A. baylyi* ADP1. The MIC profile of ACE showed that this strain is susceptible to many antimicrobial agents recommended for clinical treatment of *Acinetobacter* spp.

All these results provide a valuable reference for future genomic, metabolic and host–microbe interaction studies to broaden the knowledge of the biology of *Acinetobacter*. In particular, this study supports the pursuit of studies aimed at understanding the remarkable acetate catabolism of ACE, including efforts towards its use in microbiological and biotechnological applications. For example, a strain like ACE, which catabolizes acetate exclusively, could be used for the microbiological removal of this acid from high-density cultures of *E. coli* or other production strains [71] and for the elimination of acetate from lignocellulosic hydrolysates to abolish the inhibition of micro-organism growth used to produce biofuels and chemicals from agricultural residues [72].

Funding information

This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACyT) grant CB-2012-01/183813 (J.-C.S.) and grant CB-2010/156451 (S.L.B.); and Programa de Mejoramiento del Profesorado (PROMEP) 103.5/12/3530/UAM-PTC-302 (J.-C.S.).

Acknowledgements

We acknowledge Professor Veronique de Berardinis (Genoscope CNS, France) for kindly donating the *Acinetobacter baylyi* ADP1 strain used in this study. The authors also thank Dr Jesús Silva (Instituto Nacional de Salud Pública, Mexico) and Dr Gabriel Viguera (Universidad Autónoma Metropolitana, Mexico) for their technical support.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Towner K. The genus *Acinetobacter*. *Prokaryotes* 2006;6:746–758.

2. Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. *Microbes Environ* 2011;26:101–112.
3. Metzgar D, Bacher JM, Pezo V, Reader J, Döring V et al. *Acinetobacter* sp. ADP1: an ideal model organism for genetic analysis and genome engineering. *Nucleic Acids Res* 2004;32:5780–5790.
4. Young DM, Parke D, Ornston LN. Opportunities for genetic investigation afforded by *Acinetobacter baylyi*, a nutritionally versatile bacterial species that is highly competent for natural transformation. *Annu Rev Microbiol* 2005;59:519–551.
5. Elliott KT, Neidle EL. *Acinetobacter baylyi* ADP1: transforming the choice of model organism. *IUBMB Life* 2011;63:1075–1080.
6. de Berardinis V, Durot M, Weissenbach J, Salanoubat M. *Acinetobacter baylyi* ADP1 as a model for metabolic system biology. *Curr Opin Microbiol* 2009;12:568–576.
7. Nemeč A, De Baere T, Tjernberg I, Vaneechoutte M, van der Reijden TJ et al. *Acinetobacter ursingii* sp. nov. and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. *Int J Syst Evol Microbiol* 2001;51:1891–1899.
8. Dortet L, Legrand P, Soussy CJ, Cattoir V. Bacterial identification, clinical significance, and antimicrobial susceptibilities of *Acinetobacter ursingii* and *Acinetobacter schindleri*, two frequently misidentified opportunistic pathogens. *J Clin Microbiol* 2006;44:4471–4478.
9. Carr EL, Kämpfer P, Patel BK, Gürtler V, Seviour RJ. Seven novel species of *Acinetobacter* isolated from activated sludge. *Int J Syst Evol Microbiol* 2003;53:953–963.
10. Wolfe AJ. The acetate switch. *Microbiol Mol Biol Rev* 2005;69:12–50.
11. Flores N, de Anda R, Flores S, Escalante A, Hernández G et al. Role of pyruvate oxidase in *Escherichia coli* strains lacking the phosphoenolpyruvate:carbohydrate phosphotransferase system. *J Mol Microbiol Biotechnol* 2005;8:209–221.
12. Oh MK, Rohlin L, Kao KC, Liao JC. Global expression profiling of acetate-grown *Escherichia coli*. *J Biol Chem* 2002;277:13175–13183.
13. El-Mansi M, Cozzone AJ, Shiloach J, Eikmanns BJ. Control of carbon flux through enzymes of central and intermediary metabolism during growth of *Escherichia coli* on acetate. *Curr Opin Microbiol* 2006;9:173–179.
14. Sigala JC, Flores S, Flores N, Aguilar C, de Anda R et al. Acetate metabolism in *Escherichia coli* strains lacking phosphoenolpyruvate:carbohydrate phosphotransferase system; evidence of carbon recycling strategies and futile cycles. *J Mol Microbiol Biotechnol* 2009;16:224–235.
15. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173:697–703.
16. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990;215:403–410.
17. van Dongen S, Abreu-Goodger C. Using MCL to extract clusters from networks. In: van Helden J, Toussaint A and Thieffry D (editors). *Bacterial Molecular Networks: Methods and Protocols*. New York, NY: Springer New York; 2012. pp. 281–295.
18. Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc Bioinformatics* 2002;Chapter 2:Unit 2.3.
19. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
20. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 2008;18:821–829.
21. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. Scaffold-1ng pre-assembled contigs using SSPACE. *Bioinformatics* 2011;27:578–579.
22. Gordon D, Abajian C, Green P. *Consed*: a graphical tool for sequence finishing. *Genome Res* 1998;8:195–202.
23. Delcher AL, Bratke KA, Powers EC, Salzberg SL. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 2007;23:673–679.
24. Carver T, Harris SR, Berriman M, Parkhill J, McQuillan JA. Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. *Bioinformatics* 2012;28:464–469.
25. Deng Y, Li J, Wu S, Zhu Y, Chen YHF. Integrated nr database in protein annotation system and its localization. *Comput Eng* 2006;32:71–74.
26. Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F et al. CDD: NCBI's conserved domain database. *Nucleic Acids Res* 2015;43:D222–D226.
27. Mitchell A, Chang HY, Daugherty L, Fraser M, Hunter S et al. The InterPro protein families database: the classification resource after 15 years. *Nucleic Acids Res* 2015;43:D213–D221.
28. Siguié P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 2006;34:D32–D36.
29. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
30. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M et al. Versatile and open software for comparing large genomes. *Genome Biol* 2004;5:R12.
31. Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M. The KEGG resource for deciphering the genome. *Nucleic Acids Res* 2004;32:D277–D280.
32. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*; Twentieth Informational Supplement. CLSI document M100–S20. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
33. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*; Approved Standard—Ninth Edition. CLSI document M07–A9; 2012.
34. Fondi M, Bacci G, Brilli M, Papaleo MC, Mengoni A et al. Exploring the evolutionary dynamics of plasmids: the *Acinetobacter* pan-plasmidome. *BMC Evol Biol* 2010;10:59.
35. Chan JZ, Halachev MR, Loman NJ, Constantinidou C, Pallen MJ. Defining bacterial species in the genomic era: insights from the genus *Acinetobacter*. *BMC Microbiol* 2012;12:302.
36. Juni E. Genetics and physiology of *Acinetobacter*. *Annu Rev Microbiol* 1978;32:349–371.
37. de Berardinis V, Vallenet D, Castelli V, Besnard M, Pinet A et al. A complete collection of single-gene deletion mutants of *Acinetobacter baylyi* ADP1. *Mol Syst Biol* 2008;4:174.
38. Vaneechoutte M, Young DM, Ornston LN, de Baere T, Nemeč A et al. Naturally transformable *Acinetobacter* sp. strain ADP1 belongs to the newly described species *Acinetobacter baylyi*. *Appl Environ Microbiol* 2006;72:932–936.
39. Harwood CS, Parales RE. The β -ketoadipate pathway and the biology of self-identity. *Annu Rev Microbiol* 1996;50:553–590.
40. Fischer R, Bleichrodt FS, Gerischer UC. Aromatic degradative pathways in *Acinetobacter baylyi* underlie carbon catabolite repression. *Microbiology* 2008;154:3095–3103.
41. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol* 2011;9:244–253.
42. Lewis JA, Horswill AR, Schwem BE, Escalante-Semerena JC. The Tricarballoylate utilization (*tcuRABC*) genes of *Salmonella enterica* serovar Typhimurium LT2. *J Bacteriol* 2004;186:1629–1637.
43. Durot M, Le Fèvre F, de Berardinis V, Kreimeyer A, Vallenet D et al. Iterative reconstruction of a global metabolic model of *Acinetobacter baylyi* ADP1 using high-throughput growth phenotype and gene essentiality data. *BMC Syst Biol* 2008;2:85.
44. Barbe V, Vallenet D, Fonknechten N, Kreimeyer A, Oztas S et al. Unique features revealed by the genome sequence of

- Acinetobacter* sp. ADP1, a versatile and naturally transformation competent bacterium. *Nucleic Acids Res* 2004;32:5766–5779.
45. Lin E. et al. Dissimilatory Pathways for Sugars, Polyols, and Carboxylates. In: Neidhardt FC, Curtiss R III, Ingraham JL, Lin ECC, Low KB et al. (editors). *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology*, 2nd ed, vol. 1. Washington, DC: ASM Press; 1996. pp. 307–342.
 46. Imperi F, Antunes LC, Blom J, Villa L, Iacono M et al. The genomics of *Acinetobacter baumannii*: insights into genome plasticity, antimicrobial resistance and pathogenicity. *IUBMB Life* 2011;63:1068–1074.
 47. Antunes LC, Visca P, Towner KJ. *Acinetobacter baumannii*: evolution of a global pathogen. *Pathog Dis* 2014;71:292–301.
 48. Cerqueira GM, Peleg AY. Insights into *Acinetobacter baumannii* pathogenicity. *IUBMB Life* 2011;63:1055–1060.
 49. Adams MD, Goglin K, Molyneaux N, Hujer KM, Lavender H et al. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. *J Bacteriol* 2008;190:8053–8064.
 50. Poirel L, Bonnin RA, Nordmann P. Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. *IUBMB Life* 2011;63:1061–1067.
 51. Nikaido H, Takatsuka Y. Mechanisms of RND multidrug efflux pumps. *Biochim Biophys Acta* 2009;1794:769–781.
 52. Mcgann P, Milillo M, Clifford RJ, Snesrud E, Stevenson L et al. Detection of New Delhi metallo- β -lactamase (encoded by bla_{NDM-1}) in *Acinetobacter schindleri* during routine surveillance. *J Clin Microbiol* 2013;51:1942–1944.
 53. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β -lactamases. *Antimicrob Agents Chemother* 2010;54:24–38.
 54. Beceiro A, Fernández-Cuenca F, Ribera A, Martínez-Martínez L, Pascual A et al. False extended-spectrum β -lactamase detection in *Acinetobacter* spp. due to intrinsic susceptibility to clavulanic acid. *J Antimicrob Chemother* 2008;61:301–308.
 55. Antunes LC, Imperi F, Carattoli A, Visca P. Deciphering the multifactorial nature of *Acinetobacter baumannii* pathogenicity. *PLoS One* 2011;6:e22674.
 56. Antunes LC, Imperi F, Towner KJ, Visca P. Genome-assisted identification of putative iron-utilization genes in *Acinetobacter baumannii* and their distribution among a genotypically diverse collection of clinical isolates. *Res Microbiol* 2011;162:279–284.
 57. Cartron ML, Maddocks S, Gillingham P, Craven CJ, Andrews SC. Feo – transport of ferrous iron into bacteria. *Biometals* 2006;19:143–157.
 58. Stintzi A, Barnes C, Xu J, Raymond KN. Microbial iron transport via a siderophore shuttle: a membrane ion transport paradigm. *Proc Natl Acad Sci USA* 2000;97:10691–10696.
 59. Mihara K, Tanabe T, Yamakawa Y, Funahashi T, Nakao H et al. Identification and transcriptional organization of a gene cluster involved in biosynthesis and transport of acinetobactin, a siderophore produced by *Acinetobacter baumannii* ATCC 19606^T. *Microbiology* 2004;150:2587–2597.
 60. Noinaj N, Guillier M, Barnard TJ, Buchanan SK. TonB-dependent transporters: regulation, structure, and function. *Annu Rev Microbiol* 2010;64:43–60.
 61. Choi CH, Lee EY, Lee YC, Park TI, Kim HJ et al. Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. *Cell Microbiol* 2005;7:1127–1138.
 62. McConnell MJ, Actis L, Pachón J. *Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models. *FEMS Microbiol Rev* 2013;37:130–155.
 63. Jacobs AC, Hood I, Boyd KL, Olson PD, Morrison JM et al. Inactivation of phospholipase D diminishes *Acinetobacter baumannii* pathogenesis. *Infect Immun* 2010;78:1952–1962.
 64. Choi AH, Slamti L, Avci FY, Pier GB, Maira-Litrán T. The pgaABCD locus of *Acinetobacter baumannii* encodes the production of poly- β -1-6-*N*-acetylglucosamine, which is critical for biofilm formation. *J Bacteriol* 2009;191:5953–5963.
 65. Di Nocera PP, Rocco F, Giannouli M, Triassi M, Zarrilli R. Genome organization of epidemic *Acinetobacter baumannii* strains. *BMC Microbiol* 2011;11:224.
 66. Mortensen BL, Skaar EP. Host-microbe interactions that shape the pathogenesis of *Acinetobacter baumannii* infection. *Cell Microbiol* 2012;14:1336–1344.
 67. Smith MG, Gianoulis TA, Pukatzki S, Mekalanos JJ, Ornston LN et al. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. *Genes Dev* 2007;21:601–614.
 68. Abbott BJ, Laskin AI, McCoy CJ. Growth of *Acinetobacter calcoaceticus* on ethanol. *Appl Microbiol* 1973;25:787–792.
 69. Ensley BD, Finnerty WR. Influences of growth substrates and oxygen on the electron transport system in *Acinetobacter* sp. H01-N. *J Bacteriol* 1980;142:859–868.
 70. Preez JC, Toerien DF, Lategan PM. Growth parameters of *Acinetobacter calcoaceticus* on acetate and ethanol. *Eur J Appl Microbiol Biotechnol* 1981;13:45–53.
 71. Santala S, Karp M, Santala V. Rationally engineered synthetic coculture for improved biomass and product formation. *PLoS One* 2014;9:e113786.
 72. Kannisto MS, Mangayil RK, Shrivastava-Bhattacharya A, Pletschke BI, Karp MT et al. Metabolic engineering of *Acinetobacter baylyi* ADP1 for removal of *Clostridium butyricum* growth inhibitors produced from lignocellulosic hydrolysates. *Biotechnol Biofuels* 2015;8:198.
 73. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*, Twenty-Fifth Informational Supplement. CLSI document M100-S25 ; 2015.

Edited by: P. Langford and M. Whiteley

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