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.

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*,...
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 environ-
mental 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 stud-
ied 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 inter-
est 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 corre-
late the phenotypic characteristics observed under labora-
tory conditions with the metabolic pathways predicted from
the genome. We also attempted to identify factors that
would contribute to the knowledge of the possible interac-
tions 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 µg ml⁻¹) [14]. Single colonies were obtained by streak-
ning on Luria–Bertani (LB) plates with kanamycin (30 µg
ml⁻¹) and used to inoculate M9 liquid mineral medium
supplemented with 3 g sodium acetate l⁻¹ and 30 µg kana-
mycin ml⁻¹. Once in the exponential phase, samples were
collected from the liquid culture and streaked on LB plates
with kanamycin (30 µg 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 iso-
lates retrieved in this medium was approximately 0.9 h⁻¹.
This value was used as a parameter to distinguish the colo-
nies 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 phylo-
genomic tree, all the genes of 14 Actinobacter 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 PROTGAMMAWAG 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 ×. Library construc-
tion 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], Space-
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) with MUMmer was
calculated using the JSpecies software [29]. The DNA con-
servation 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 spe-
cific protein families between different Actinobacter 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 g Na₂HPO₄
1−1, 0.5 g NaCl 1−1, 3 g KH₂PO₄ 1−1, 1 g NH₄Cl 1−1,
240.9 mg MgSO₄ 1−1, 11.1 mg CaCl₂ 1−1, 0.02 mg FeSO₄.
.7H₂O ml⁻¹ and 2.0 mg vitamin B₁ 1−1. Frozen vials stored at
−80 °C with glycerol (25 %, v/v) were used to start the inocula-
on LB broth (5 g yeast extract 1−1, 10 g tryptone 1−1, 10 g NaCl
1−1) 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 g sodium acetate
1−1 for 12 to 18 h at 30 °C and an agitation speed of
250 r.p.m. ACE cultures were supplemented with 30 µg kana-
mycin ml⁻¹. Bioreactor cultivations were performed in a 11
BiostabT Plus bioreactor (Sartorius) containing 0.60 l
M9 medium supplemented with 5.5 g sodium acetate 1−1
and inoculated at an initial optical density of 0.1 at 600 nm
(OD₆₀₀ 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 OD600nm=0.55 gDCW 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 correlating absorbance at 600 nm (Genesys 20, Thermo Scientific). For bioreactor Cultivars, 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 OD600nm=0.55 gDCW 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).

RESULTS AND DISCUSSION

Discovery of the ACE strain

In the course of experimental study of an E. coli mutant, strain PB12 maeB::lxa [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::lxa, 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 NIP0100 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 Iwofii 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 extrachromosomal elements.
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-ketoadipate 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
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).
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 tricarballylate or related compounds such as citrate, *cis*-aconitate or isocitrate.
ACE, but the protocatechuate branch is incomplete owing to the absence of pcaBH genes (Fig. 4b). PcaBH are protocatechuate oxygenases that produce β-carboxymuconate, which is transformed to γ-carboxymuconalactone by the cycloisomerase PcaB. This carboxymuconalactone 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 pcaUFBKDHG-aroD-pobRA-hcaGECA-mdcABCDEGHLMR (cluster IV) (Fig. S2).

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Acinetobacter strain</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
</tr>
<tr>
<td>Succinate</td>
<td>+/-</td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
</tr>
<tr>
<td>PHB</td>
<td>–</td>
</tr>
<tr>
<td>Vanillate</td>
<td>–</td>
</tr>
<tr>
<td>Malate</td>
<td>–</td>
</tr>
<tr>
<td>Acetate</td>
<td>+</td>
</tr>
<tr>
<td>Lactate</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>–</td>
</tr>
<tr>
<td>Xylose</td>
<td>–</td>
</tr>
<tr>
<td>Arabinose</td>
<td>–</td>
</tr>
</tbody>
</table>

*+, Growth; +/-, slight growth; –, no growth. Results determined after 24 h of incubation at 30°C and 250 rpm. 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.

[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 (gpK) 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.
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), 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
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. lwofii 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 Fe$^{2+}$ 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_CH01726 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 pgaABC (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 CsaU/BABCD chaperone-usher pilus assembly system, biofilm-associated protein (Bap), AbaI autoinducer synthase, two-component regulatory system (BfiRS), 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.
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 $\mu$ of 0.89 h⁻¹ and ADP1 had a $\mu$ of 0.44 h⁻¹ (Table 4). Additional cultivation parameters showed that the biomass yield of ACE on acetate (Y<sub>x/s</sub>) 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 $\mu$ 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<sub>max</sub> 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>
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<tr>
<th>Description</th>
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<th>Best blastp match with <em>A. baumannii</em> sequences</th>
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<td></td>
<td></td>
<td>Identity (%)</td>
<td>Amino acids</td>
</tr>
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<td>Antibiotic resistance</td>
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<td>81</td>
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<tr>
<td>AsACE_p300003</td>
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<td>Fe(II) iron uptake systems</td>
<td>Ferrous iron transporter, FeoA</td>
<td>AsACE_CH02668</td>
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<tr>
<td>FeoA</td>
<td>AsACE_p100053</td>
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<td>97</td>
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<td>Ferrous iron transporter, FeoB</td>
<td>AsACE_CH02667</td>
<td>618</td>
<td>85</td>
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<tr>
<td>FeoC</td>
<td>NF</td>
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<td>3</td>
<td>Fe(III)-loaded siderophores</td>
<td>TonB family transporter</td>
<td>AsACE_CH00410</td>
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<td>Transporter ExbB/TolQ 1</td>
<td>AsACE_CH02443</td>
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<td>AsACE_CH01727</td>
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<td>AsACE_CH01944</td>
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<td>84</td>
<td>232</td>
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<tr>
<td>Transporter ExbD/TolR 1</td>
<td>AsACE_CH00412</td>
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<td>96</td>
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<tr>
<td>AsACE_CH01726</td>
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<td>AsACE_CH01945</td>
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<td>150</td>
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<td>Haem oxygenase (<em>hemO</em>)</td>
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<td>Acinetobactin synthesis and transport proteins</td>
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<td>4</td>
<td>Outer membrane protein A (OmpA)</td>
<td>AsACE_CH02243</td>
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<td>5</td>
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<td>AsACE_CH02260</td>
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<td>AsACE_CH01516</td>
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<td>69</td>
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<td>6</td>
<td>Phospholipase C</td>
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<td>7</td>
<td>Capsule formation (<em>ptk</em> and <em>epsA)</em></td>
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</tr>
<tr>
<td>8</td>
<td>Extracellular polysaccharide</td>
<td>Poly-N-acetylgulcosamine (PNAG) export porin PgaA</td>
<td>AsACE_CH000825</td>
</tr>
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<td>AsACE_CH000826</td>
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<td>45</td>
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<tr>
<td>PNAG export synthase PgaC</td>
<td>AsACE_CH000827</td>
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<td>73</td>
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<tr>
<td>PNAG biosynthesis PgaD</td>
<td>AsACE_CH000828</td>
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<td>48</td>
</tr>
<tr>
<td>PNAG domain-containing protein</td>
<td>AsACE_CH000829</td>
<td>400</td>
<td>39</td>
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</table>

NF, not found.
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 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 gluconeogetic 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].

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

These data come from two independent cultures and MIC (µg ml⁻¹) determinations. MIC interpretive standards for Acinetobacter spp. are shown according to the Clinical and Laboratory Standards Institute [73] unless otherwise established.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC of ACE</th>
<th>Interpretation</th>
<th>MIC range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&lt;=8</td>
<td>NA</td>
<td>&lt;=8, 16, &gt;=32²a</td>
</tr>
<tr>
<td>Ampicillin/</td>
<td>&lt;=8/4</td>
<td>S</td>
<td>&lt;=8/4, 16/8, &gt;=32/</td>
</tr>
<tr>
<td>sulbactam</td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Ticarcillin/</td>
<td>&lt;=16</td>
<td>S</td>
<td>&lt;=16/2, 32/2–64/</td>
</tr>
<tr>
<td>clavulanate</td>
<td></td>
<td></td>
<td>2, &gt;=128/2</td>
</tr>
<tr>
<td>Cepheps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazoline</td>
<td>&gt;16</td>
<td>NA</td>
<td>&lt;=2, 4, &gt;=8⁵</td>
</tr>
<tr>
<td>Cefepine</td>
<td>3</td>
<td>S</td>
<td>&lt;=8, 16, &gt;=32</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>8</td>
<td>S</td>
<td>&lt;=8, 16–32, &gt;=64</td>
</tr>
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<td>Cefotaxime/</td>
<td>&lt;=0.5</td>
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<td>NA</td>
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<td>clavulanate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotetan</td>
<td>32</td>
<td>NA</td>
<td>&lt;=16, 32, &gt;=64⁴b</td>
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<tr>
<td>Cefazidime</td>
<td>4</td>
<td>S</td>
<td>&lt;=8, 16, &gt;=32</td>
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<td>Cefazidime/</td>
<td>&lt;=0.25</td>
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<td>NA</td>
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<tr>
<td>clavulanate</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&lt;=8</td>
<td>S</td>
<td>&lt;=8–16, &gt;=32</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>6</td>
<td>S</td>
<td>&lt;=8, 16, &gt;=32²b</td>
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<tr>
<td>Monobactams</td>
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<td>Aztreonam</td>
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<td>&lt;=8, 16, &gt;=32²</td>
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<td>Meropenem</td>
<td>&lt;=4</td>
<td>I</td>
<td>&lt;=2, 4, &gt;=8</td>
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<td>Aminocyclotols</td>
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<tr>
<td>Spectinomycin</td>
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<td>&lt;=32, 64, &gt;=128⁸</td>
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<td>&lt;=4, 8, &gt;=16</td>
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<tr>
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<td>&lt;=16, 32, &gt;=64⁴d</td>
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<td>NA</td>
</tr>
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<td>&lt;=1</td>
<td>S</td>
<td>&lt;=4, 8, &gt;=16</td>
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<tr>
<td>Folate pathway</td>
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<tr>
<td>inhibitors</td>
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<tr>
<td>Trimethoprim/</td>
<td>&lt;=2/38</td>
<td>S</td>
<td>&lt;=2/38, &gt;=4/76</td>
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<td>sulfamethoxazole</td>
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<td>S</td>
<td>&lt;=1, 2, &gt;=4</td>
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<td>Levofloxacin</td>
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<td>S</td>
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<tr>
<td>Moxifloxacin</td>
<td>&lt;=2</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

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.

Table 4. Specific rates of growth (µ) and acetate consumption (qAce) calculated during exponential growth, and cell mass yield on consumed acetate (Ys/C0) calculated at the depletion of acetate

Experiments were performed in bioreactors and using 5.5 g acetate [11]. ±, Standard error of duplicate experiments.

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Acinetobacter strain</th>
<th>ADP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>µ (h⁻¹)</td>
<td>0.89±0.06</td>
<td>0.44±0.01</td>
</tr>
<tr>
<td>Ys/C0 (gDCW gAce⁻¹)</td>
<td>0.43±0.06</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>qAce (gAce gDCW h⁻¹)</td>
<td>2.06±0.10</td>
<td>2.39±0.07</td>
</tr>
</tbody>
</table>

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## Conflicts of interest
The authors declare that they have no conflicts of interest.

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