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Identification of virulence markers in clinically relevant strains of *Acinetobacter* genospecies

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Summary. Nine *Acinetobacter* strains from patients and hospital environment were analyzed for virulence markers, quorum sensing signal production, and the presence of *luxI* and *luxR* genes. The strains had several properties in common: growth in iron limited condition, biofilm formation, and no active protease secretion. Significantly higher catechol production was determined in patient isolates ($P < 0.03$), but other invasiveness markers, such as lipase secretion, amount of biofilm, cell motility, antibiotic resistance, and hemolysin production, showed large variability. Notably, all members of the so-called *A. calcoaceticus-A. baumannii* complex, regardless of whether the source was a patient or environmental, secreted medium- to long-chain *N*-acyl homoserine lactones (AHL) and showed blue light inhibition of cell motility. In these strains, a *luxI* homologue with a homoserine lactone synthase domain and a *luxR* putative regulator displaying the typical AHL binding domain were identified. [**Int Microbiol** 2012; 15(2):79-88]

Keywords: *Acinetobacter* · light · phylogeny · quorum sensing · virulence markers

Introduction

Acinetobacter spp. represent a well defined genus of gram-negative non-fermentative bacteria from the Gammaproteobacteria class. The taxonomy of the genus *Acinetobacter* has undergone extensive revision; to date, 23 named species and 11 species with provisional designations have been described [7,30]. Four species of this genus, referred to as the *A. calcoaceticus-A. baumannii* complex, form a closely related group of glucose-acidifying strains, difficult to distinguish from each other by phenotypic tests: *A. calcoaceticus*, *A. baumannii*, *A. pittii* (formerly *Acinetobacter* genomic species 3), and *A. nosocomialis* (formerly *Acinetobacter* genomic species 13TU) [11,20]. The group includes the most clinically relevant species due to their ability to cause nosocomial outbreaks and to acquire antibiotic resistance [3,4,16]. Clinical isolates of these bacteria are frequently resistant to major antibiotics and are able to survive for long periods in a hospital environment, which may be advantageous for their persistence [25]. Nevertheless, other attributes, such as attachment to mucosal tissues, proliferation, and avoidance of the immune system in order to trigger invasion are also critical in causing disease. The production of fimbriae (pili), enzymes, toxins and iron chelators may contribute significantly to the success of infection. In addition, the expression

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of these factors can be modified by environmental conditions, such as medium composition, pH, temperature, osmolarity, and population dynamics within the bacterial community [7,8,33].

Quorum sensing (QS) regulates the expression of virulence factors in many gram-negative bacteria [23]. *Acinetobacter* strains have been shown to produce quorum signals of the *N*-acyl homoserine lactone (AHL) type, affecting biofilm formation in *A. baumannii* strain M2 [21]. Nevertheless, the contribution of quorum sensors to the overall pathogenic potential in the genus is currently unknown.

In a previous work [12], we analyzed the pattern of AHLs produced in 43 strains belonging to 20 classified and unclassified genomic *Acinetobacter* species. The AHL synthases and their receptors/regulators constitute evolutionarily conserved protein families known as LuxI and LuxR, respectively. In this study, nine of the nosocomial strains were selected for a detailed analysis of virulence-related phenotypic markers as well as a new assessment of QS signals using another detection system, and the search for synthase and regulator genes (*luxI* and *luxR)* along with their phylogenetic analysis. Our results may help in identifying common features in opportunistic strains that could yield insights into new ways to tackle invasion.

Materials and methods

Strains and culture conditions. The strains used in this study are listed in Table 1. *Acinetobacter* isolates were obtained from the L. Dijkshoorn collection, Leiden University Medical Center, Leiden, the Netherlands. The strains had been previously identified by DNA–DNA hybridization [29] or amplified ribosomal DNA restriction analysis by amplified fragment length polymorphism (AFLP) analysis [15]. Unless otherwise stated, *Acinetobacter* strains were grown overnight at 30 ºC in lysogeny broth (LB). *Agrobacterium tumefaciens* NT1/pZLR4 was grown in glucose M9 broth containing 30 µg gentamycin /ml to mid exponential phase at 29 °C. The cells were centrifuged at 12000 ×*g* for 15 min at 4 °C, and the pellet was resuspended in 1/20 of the original culture volume in the same medium containing 20 % (v/v) glycerol. This suspension was stored in 1-ml cryovials at –80 °C until further use. *Chromobacterium violaceum* CV026 was grown in LB containing 25 µg kanamycin/ml at 28 ºC. All the strains were incubated with agitation at 215 rpm.

Lipase and protease activities. Lipase activity was measured in culture supernatants as previously described [17] using *p*-nitrophenyl palmi-

Table 1. Bacterial strains used in the study

Strain*	Species	Source / description	Origin	Reference
RUH 0584	*Acinetobacter calcoaceticus*	Soil	Rotterdam NL	[15]
RUH 0875	*A. baumannii*	Urine	Rotterdam NL	
RUH 0134	*A. baumannii*	Urine	Rotterdam NL	
RUH 0509	*A. pittii*	Bronchus	Nijmegen NL	
RUH 0503	*A. nosocomialis*	Urine	Nijmegen NL	
RUH 0044	*A. haemolyticus*	Air	Rotterdam NL	
RUH 0204	*A. junii*	Blood	Rotterdam NL	[29]
RUH 0045	*A. lwoffii*	Blood	Rotterdam NL	[15]
RUH 3517	*A. radioresistens*	Pillow	Hengelo NL	
NT1/pZLR4	*Agrobacterium tumefaciens*	Biosensor strain for detection of AHLs with medium to long length acyl chain†	SILS Institute‡	[5]
CV 026	*Chromobacterium violaceum*	Biosensor strain for the detection of AHLs with short length acyl chain§	SILS Institute‡	[18]

*RUH strains from L. Dijkshoorn collection, Leiden University Medical Center, Leiden, the Netherlands.
†Strain used for detection of all 3-oxo AHL; C_6-C_{14}AHL; 3-OH-C_6AHL; 3-OH-C_8AHL and 3-OH-C_{10}AHL [9].
‡Swammerdam Institute for Life Sciences, University of Amsterdam, the Netherlands.
§Strain used for detection of C_4-C_8 AHL, 3-oxo-C_6 AHL and 3-oxo-C_8 AHL.

tate (pNPP) as the substrate. The molar absorption coefficient of *p*-nitrophenol in the assay buffer at pH 8.0 was determined to be 15,100 $M^{-1}cm^{-1}$. One unit of enzyme activity is defined as the amount of enzyme forming 1 μmol min^{-1} pNP. Proteolytic activity was measured according to the method previously described [32], except that the incubation temperature was 30 ºC.

Biofilm assay. *Acinetobacter* strains were grown in shaking flasks in LB medium overnight at 30 ºC at 215 rpm. Cultures were diluted 1/100 with fresh LB medium and inoculated on polystyrene 96-well plates using 200 μl of the cell suspension. After a 24 h incubation of the cultures at 30 °C, 50 μl was removed from each well, diluted with 150 μl of LB, and the optical density at 600 nm was measured. The remaining samples were carefully removed, after which the wells were gently washed three times with 200 μl of PBS, dried in an inverted position, and stained with 1% (w/v) crystal violet (CV) for 20 min. The wells were then washed five times with 400 μl of PBS, the CV-stained cells were solubilized in 200 μl of ethanol, and the samples incubated for 30 min with gentle mixing (80 rpm). The OD_{587} of the solubilized biofilm was measured and the results were expressed as OD_{587}/OD_{600}. A blank was processed under the same conditions using LB medium. The assay was performed twice and each strain was assessed in a 96-well plate. Results were expressed as mean ± SD.

Hemolysis. *Acinetobacter* strains were streaked on Columbia agar plates (Bio Merieux) supplemented with 5 % v/v sheep blood and incubated at 30 ºC for 48 h. Hemolysis was observed as a clear zone around the colonies.

Antibiotic resistance assay. Antibiotic susceptibility was tested with the Kirby-Bauer-disk diffusion method [2], using the CLSI protocol. Antibiotics selected for this analysis were gentamicin, ceftazidime, netilmicin, tobramycin, ofloxacin, tetracycline, ampicillin-sulbactam, piperacillin, imipenem, cotrimoxazole, meropenem and amikacin. All strains were tested for their antibiotic resistance profile and included in one of the following categories: S, sensitive to all antibiotics and MR, resistant to more than two antibiotics.

Catechol assay. Strains were grown at 30 ºC in LB broth or LB containing 400 μM ethylenediamine-di-(*o*-hydroxyphenyl) acetic acid (EDDHA), at 250 rpm for 16 h. Catechol compounds in culture supernatants were measured according to Arnow [1] as described previously [22]. Experiments were performed in duplicate and the results were analyzed by ANOVA (n = 18).

Cell motility. Cell motility was assayed as previously described [19]. Briefly, Petri plates were prepared with swimming medium containing 10 g tryptone/l, 5 g NaCl/l and 0.3 % (w/v) agarose. The plates surfaces were inoculated with bacteria from an overnight LB agar culture either using sterile wooden sticks or by depositing 3 μl of freshly grown LB cultures (OD_{600} = 0.3). The plates were incubated at 24 °C for 20–24 h in the dark or under a blue-light-emitting diode (LED) with an intensity of 5–7 μmol m^{-2} s^{-1} and an emission peak centered at 456 nm, determined using a LI-COR LI-1800 spectroradiometer. All experiments were performed in triplicate.

Short-chain AHL detection. LB plates were cross streaked with an overnight culture of the *Acinetobacter* strain to be tested and a stock suspension of *C. violaceum* CV026.

AHL detection on thin layer chromatography (TLC) plates. Samples were prepared from overnight cultures in LB broth supplemented with 0.5 % (w/v) glucose. Cultures were centrifuged at 10,000 rpm at 4 °C for 10 min and the pH of supernatants was adjusted to 5.0, prior to extraction (twice) with an equal volume of methylene chloride. The fractions were pooled, dried with anhydrous sodium sulfate, resuspended in 5 ml methanol and evaporated under a stream of nitrogen. Extracts were dissolved in methanol and analyzed on 7.5 × 5 cm TLC Silicagel 60 RP-18 F_{254}s plates (Merck KGaA, Darmstadt, Germany) using methanol:water (60:40) as the mobile phase. Each plate was also seeded with an extract of non-inoculated culture medium as a control and 2 μl of 22 mM 3-HO-C_{12}AHL or 3-oxo-C_{12}AHL in acetonitrile as reference. After drying, the plates were covered with 100 ml of a suspension prepared with 1 ml stock solution of *A. tumefaciens* NT1/pZLR4 in 1% (w/v) agarized M9 medium also containing 40 μg X-gal/ml. The plates were incubated at 29 °C for 16–24 h.

Detection of *luxI* and *luxR* type coding sequences in *Acinetobacter*. Genomic DNA of *Acinetobacter* strains was extracted with Illustra blood genomic Prep Mini Spin Kit (GE Healthcare, Buckinghamshire, UK). PCR amplifications were performed with Taq DNA polymerase (GoTaq, Promega, Madison, Wisconsin, USA) using primers designed through a consensus of ten sequences of *Acinetobacter* genomes for *luxI* and nine sequences for *luxR*, all of them deposited in the NCBI database (the sequence alignments are available by request from the corresponding author). *luxI* was amplified using primers *luxI* PF (5´-GGTTGGGAG TTGAACTGTCC-3´) and *luxI* PR (5´-AAACGTTCTACTCCAAGAGG-3´); *luxR* was amplified using primers *luxR* PF (5´-TCGGATTTGATTATTGCG CTTATG-3´) and *luxR* PR (5´-ACAGCTCGAATAGCTGCTG-3´). Amplification started with an initial denaturation step at 95 °C for 2 min followed by 30 cycles of denaturation at 95 °C, annealing at 58 °C for 45 s and extension at 72 °C for 1 min. The expected sizes of the PCR products for *luxI* and *luxR* were 370 bp and 603 bp, respectively. Nucleotide sequences of *luxI* and *luxR* genes were obtained by automated DNA sequencing. (The *luxI* and *luxR* sequences are available by request from the corresponding author.)

Phylogenetic analysis. The sequences of the putative LuxR and LuxI proteins considered in this analysis were obtained from the UniprotKB database. DNA sequences were translated into protein sequences using the Transeq at the European Bioinformatics site [http://www.ebi.ac.uk/Tools/emboss/transeq/index.html] and the sequences were aligned using Clustal W [28]. DNA sequences were compared with that of the *abaI* gene from *A. baumannii* M2, to date the only gene from *Acinetobacter* whose protein, AbaI, has been functionally characterized [21]. Neighbor-joining trees were produced with the MEGA5 program [27] using the γ model of amino acid substitution with a shape parameter a = 2.0. Confidence in neighbor-joining trees was determined by analyzing 1000 bootstrap replicates.

Results

Analysis of virulence markers. Nine *Acinetobacter* strains from patients and hospital environment were analyzed for broadly accepted virulence markers, including antibiotic resistance, biofilm formation, twitching motility in light/dark conditions, hemolysin, protease and lipase secretion and QS signals.

As a first characterization of the strains, their antibiotic resistance pattern was tested. As shown in Table 2, only *A. baumannii* and *A. nosocomialis* displayed MR, while all the others were S. All nine strains formed biofilms on abiotic surfaces. One strain, isolated from blood and typified as *A. junii* RUH 0204, had an OD_{587}/OD_{600} ratio of 26, which was significantly higher than the ratios of the other strains, which

Table 2. Virulence markers in *Acinetobacter* strains (ND, not detected)

Strain	RUH 0875	RUH 0134	RUH 0503	RUH 0204	RUH 0045	RUH 0509	RUH 0044	RUH 3517	RUH 0584
Lipase (U/OD_{580})	3.50 ± 0	0.70 ± 0	16.80 ± 4.6	ND	0.70 ± 0	16.11 ± 1.4	ND	4.9 ± 0	63.41 ± 0.4
Biofilm (OD_{587}/OD_{600})	1.69 ± 0.41	0.72 ± 0.16	0.61 ± 0.26	26.10 ± 5.47	2.33 ± 0.73	2.25 ± 0.40	2.40 ± 0.19	0.75 ± 0.26	2.99 ± 0.65
Hemolysin	–	–	–	–	–	–	+	–	–
Catechol ($\mu M/OD_{580}$)	8.07 ± 0.74	6.73 ± 0.26	2.02 ± 0.34	1.88 ± 0.5	4.69 ± 2.37	15.15 ± 9.85	1.56 ± 0.9	0.29 ± 0.41	0.62 ± 0.06
Growth at 44ºC	+	+	+	–	–	+	–	+	+
Antibiotic resistance	MR	MR	MR	S	S	S	S	S	S
Cell motility in dark	+	+	+	–	–	+	–	–	+
QS signals in TLC plates	1 spot	3 spots	1 spot	–	–	2 spots	–	–	3 spots
	Rf: 0.39	Rf: 0.06 0.20 0.41	Rf: 0.61			Rf: 0.18 0.42			Rf: 0.06 0.19 0.43

ranged from 0.61 to 2.99. Neither proteases nor short chain (C_4–C_6) AHLs were detected in any case, while hemolysis was only observed in an environmental isolate (*A. haemolyticus* RUH 0044). All of the strains were able to grow normally in iron-limited conditions, a key factor for survival in the host. Notably, strains isolated from patients secreted higher

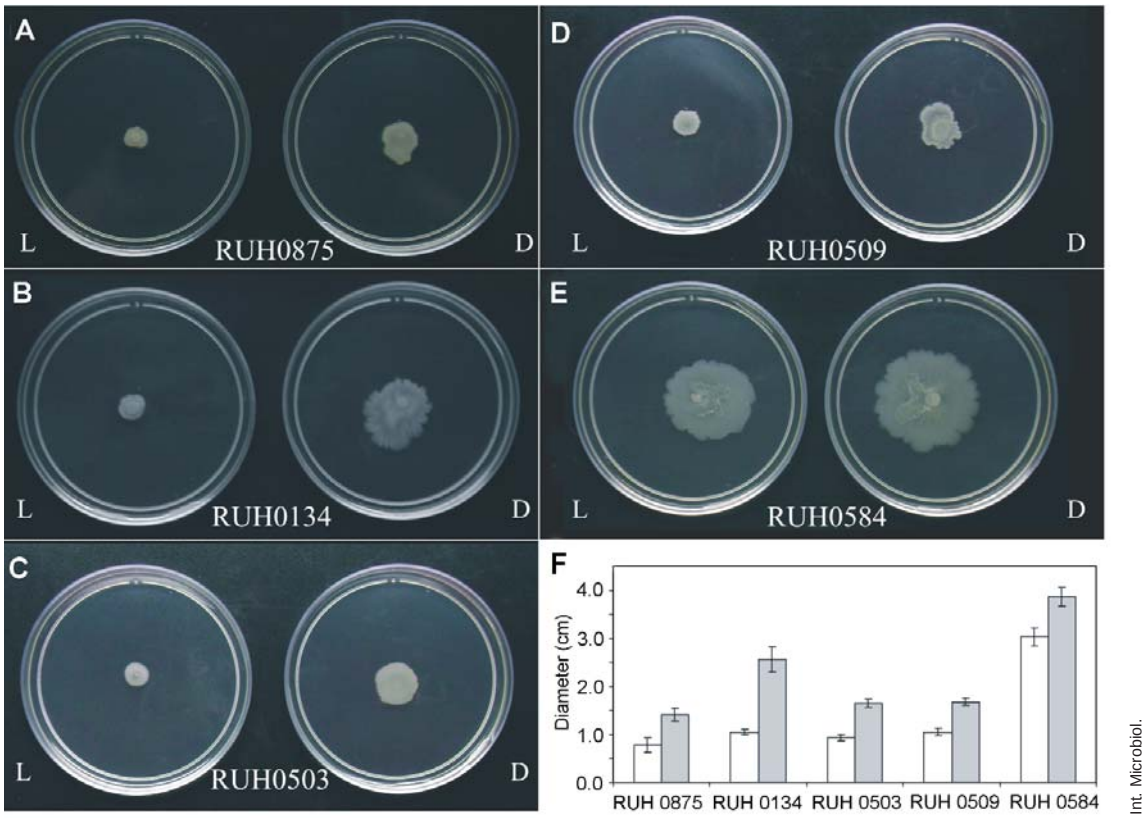

Fig. 1. Effect of light on motility. (**A**) Motility plates of *Acinetobacter baumannii* RUH 0875; (**B**) *A. baumannii* RUH 0134; (**C**) *A. nosocomialis* RUH 0503; (**D**) *A. pittii* RUH 0509; and (**E**) *A. calcoaceticus* RUH 0584, after incubation at 24 °C for 20–24 h under blue LED light (L) or in darkness (D). (**F**) Average measurements of the diameter of the cells on the motility plates shown in panels **A–E**. White bars, cells under blue LED light; dark bars, cells in darkness.

amounts of catechols than strains isolated from the environment ($P < 0.03$). Lipase activity was highest in *Acinetobacter calcoaceticus* RUH 0584, whereas the other strains, whether patient-isolated or not, produced very low levels of the enzyme.

Inhibition of cell motility by blue light was observed in all the species belonging to the *A. calcoaceticus-A. baumannii* complex, including *A. baumannii* RUH134 and RUH 875, *A. nosocomialis* RUH 0503, *A. pittii* RUH 0509 and *A. calcoaceticus* RUH 0584 (Fig. 1). Motility was not displayed by strains *A. Iwoffi* RUH 0045, *A. haemolyticus* RUH 0044 and *A. junii* RUH 0204.

Another distinctive feature of strains belonging to the *A. calcoaceticus-A. baumannii* complex was their ability to secrete medium- to long-chain AHLs. The intensity of the signals was variable; whereas signals from *Acinetobacter pittii* RUH 0509 were detected in 1-ml culture supernatant, signals from all other strains were detected using 5 to 30-ml culture supernatants. In all cases the signal molecules showed the typical behavior of lactones: they were stable at acid pH and disappeared at alkaline pH, from which they were recovered after acidification. Moreover, whereas the number of spots observed on RP-TLC plates was variable, ranging from 1 to 3, their mobility range, measured as Rf's, remained significantly unchanged (Fig. 2).

Search for *luxI* and *luxR*. To investigate these genes in the strains under study, primers were designed using a consensus of *luxI* and *luxR* sequences from 10 and 9 *Acinetobacter* genomes, respectively. All strains displaying QS signals on TLC plates yielded an amplicon of the expected size for the *luxI* and *luxR* consensus.

The percentage of identity in the *luxI* homologues varied from 85.77 % to 89.30 %, while the percentage of identity in the amino acids sequences ranged from 86.67 % to 95.56 %. Sequence analysis revealed 97.82 % identity for strain RUH 0134, 96.45 % for RUH 0875, 87.68 % for RUH 0584, 85.52 % for RUH 0509 and 84.04 % for RUH 0503. For the investigation of *luxR* type regulatory genes, the conserved AHL binding domain (pfam03472), typical of LuxR proteins, was

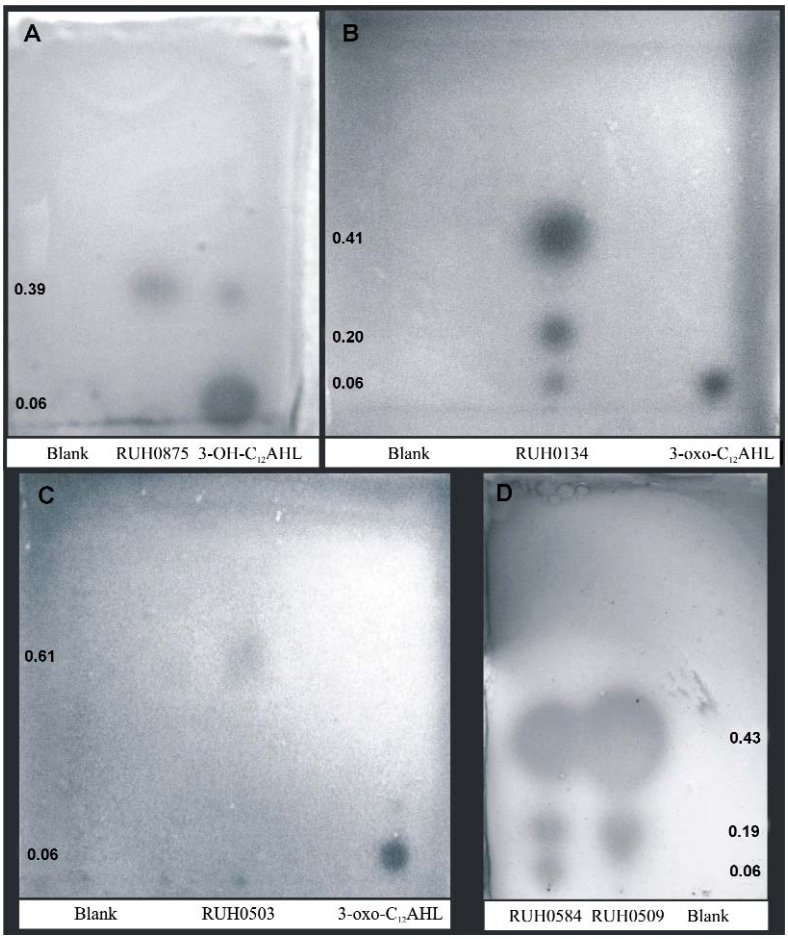

Fig. 2. AHL detection on TLC plates. RP-TLC analysis of AHL signal production by strains (**A**) *Acinetobacter baumannii* RUH 0875; (**B**) *A. baumannii* RUH 0134; (**C**) *A. nosocomialis* RUH 0503; (**D**) *A. calcoacetius* RUH 0584 and *A. pittii* RUH 0509. Each plate was also seeded with uninoculated culture medium (blank) and 2 µl of 22 mM 3-OH-C_{12}AHL, or 3-oxo-C_{12}AHL as reference.

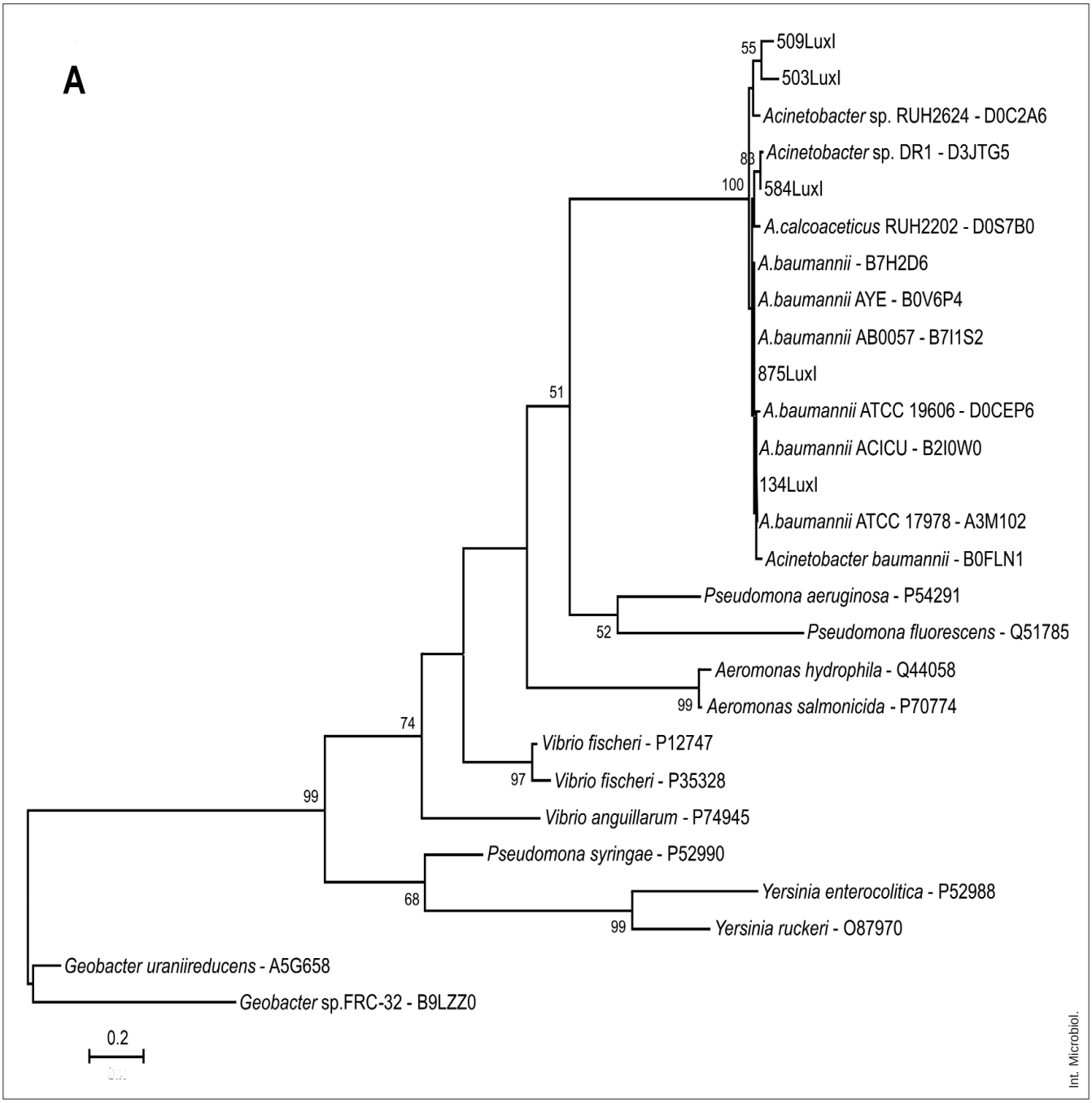

Fig. 3A. Phylogenetic tree of family members of LuxI of Gammaproteobacteria obtained by the neighbor-joining method. Sequences belonging to Deltaproteobacteria (*Geobacter*) served as outgroup. Bootstrap values as the percentage of 1000 replicates are shown for values of 50 % or higher. Species names are shown with the Uniprot accesion number. Scale bar indicates the mean number of substitutions per site.

used. The sequence identity of the *luxR* homologues was lower, varying from 85.54 % to 87.83 %, while the percentage of identity in the amino acids sequences was between 90.06 % and 93.13 %. The high percentage of amino-acid identity in the synthases and in the receptors/regulators indicates a low rate of random mutations for both genes. Note that only AHL-producing strains carried *luxI* and *luxR* type sequences, thus ruling out the presence of non-functional genes. Moreover, both markers were absent in all strains not belonging to the *A. calcoaceticus-A. baumannii* complex,

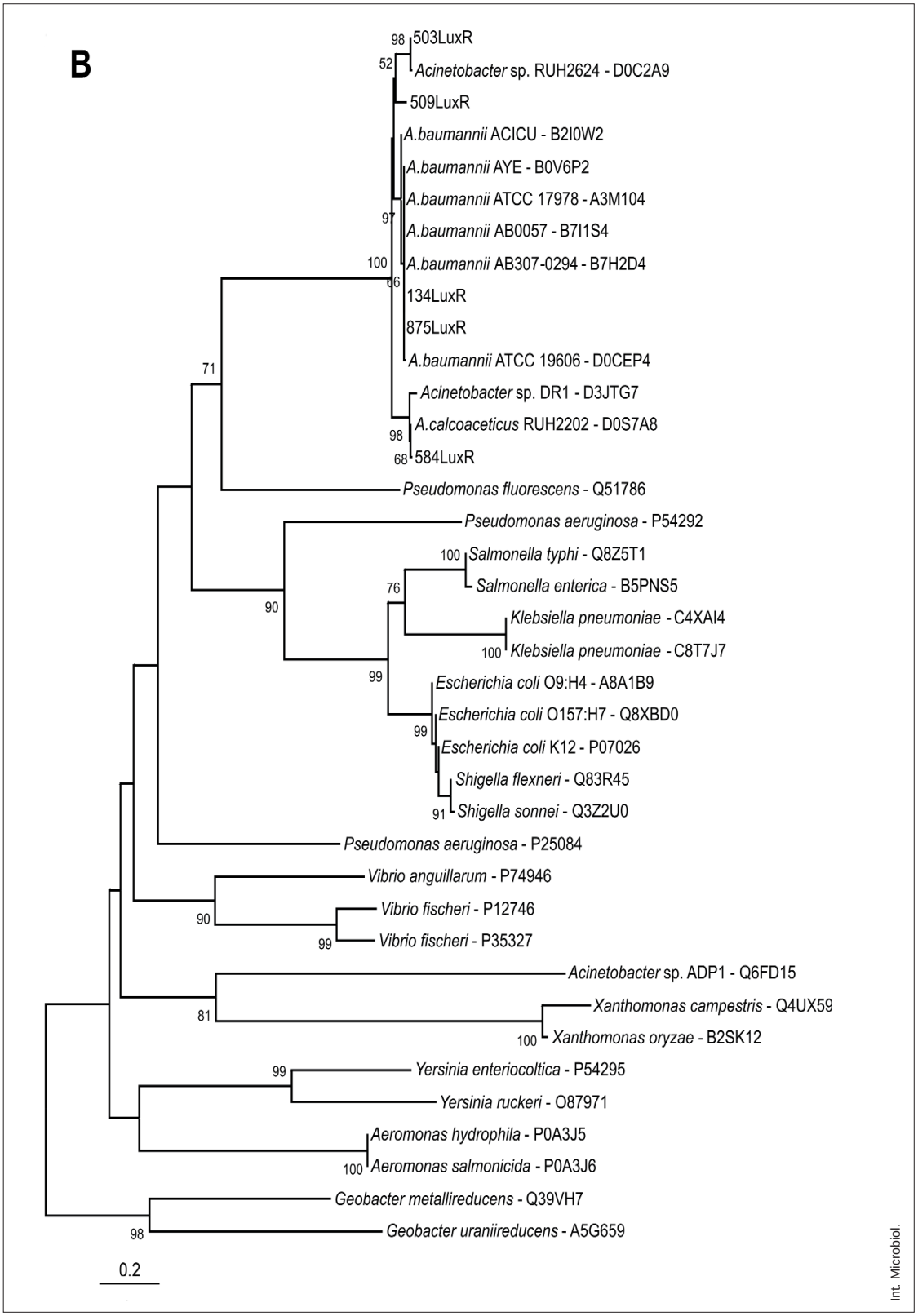

Fig. 3B. Phylogenetic tree of family members of LuxR of Gammaproteobacteria obtained by the neighbor-joining method. Sequences belonging to Deltaproteobacteria (*Geobacter*) served as outgroup. Bootstrap values as the percentage of 1000 replicates are shown for values of 50 % or higher. Species names are shown with the Uniprot accesion number. Scale bar indicates the mean number of substitutions per site.

highlighting the importance of this group with respect to QS markers.

Phylogenetic analysis of LuxI and LuxR. Figure 3 shows the results of the evolutionary analysis for the *luxI* and *luxR* translated sequences. The phylogenetic trees obtained for members of the LuxI (Fig. 3A) and LuxR (Fig. 3B) families grouped *Acinetobacter* sequences in only one cluster, except for LuxR from the environmental strain *Acinetobacter baylyi* ADP1, which is significantly separated from the *Acinetobacter* cluster. Moreover, this strain lacked any gene sequence that could be translated into a LuxI homologue. Both trees showed that strains RUH 0134 and RUH 0875 were closely related to sequences belonging to *A. baumannii*, and that strain RUH 0584 was closely related to sequences belonging to *A. calcoaceticus*, whereas other members of the *A. calcoaceticus-A. baumannii* complex (strains RUH 0509 and RUH 0503) were included in a different group. *Pseudomonas* was the most closely related genus to the *Acinetobacter* cluster and it was supported by bootstrap values of 51 % for LuxI and 71 % for LuxR. The same relationship was determined for 16S rRNA sequences (the phylogenetic tree of 16S rRNA is available by request from the corresponding author). However, the 16S rRNA phylogenetic tree grouped all *Pseudomonas* in one cluster, whereas the LuxI of *P. syringe* grouped with *Yersinia*, separated from other *Pseudomonas* LuxI homologues.

Discussion

Nosocomial outbreaks of infection caused by members of the *A. calcoaceticus-A. baumannii* complex have been increasingly reported. These reports are of particular interest since *A. baumannii* is one of the main causes of hospital acquired infections worldwide. Resistance to antibiotics and survival to desiccation are characteristics that favor *Acinetobacter* persistence in the hospital environment, but other relevant processes are needed for successful infection. For instance, a comparative genome analysis of two *A. baumannii* isolates and the non-pathogenic strain *Acinetobacter baylyi* ADP1 showed that 14 genes have a potential role in virulence [31].

In the present study, nine strains isolated from patients or clinical environment were selected from a previous work in which the pattern of QS signal production in 43 well-characterized species of *Acinetobacter* was analyzed [12]. Here, the virulence-related factors in these nine representative strains were explored. A distinctive feature of all the isolates was their ability to compete in iron-limited cultures, due to the secretion of siderophores of the catechol type. Nevertheless, differences were found between patient and environmental isolates, with significantly higher amounts of siderophores secreted by the former ($P < 0.03$). This pattern suggests the relevance of the iron uptake system as a mechanism for survival in the host, confirming a previous hypothesis [6,34]. Other responses, such as lipase secretion and biofilm formation, were also detected in most strains, but it was unclear whether these factors can be considered as infective markers for the genus. Our results agree with those of a previous work establishing that biofilm formation does not significantly differ among *Acinetobacter* species, but that the host cell response (IL-6 and IL-8) is significantly decreased upon exposure to *A. baumannii* but not to the non-clinical species *A. junii* [7].

Several studies have revealed the presence of blue-light photoreceptors in non-phototrophic organisms and their relationship to virulence. For instance, the activity of a light, oxygen, voltage photoreceptor followed by the activity of a histidine kinase from *Brucella abortus* is responsible for increased macrophage invasion [26], while a blue-light-sensing-using flavin domain from *A. baumannii* is responsible for the inhibition of cell motility and increased death of *Candida albicans* filaments [19]. As this phenomenon may be more extended than anticipated, we examined other *Acinetobacter* species by assaying cell motility in the presence of blue light or in darkness. Analysis of the light-mediated response indicated that light inhibited cell motility only in strains belonging to the *A. baumannii-A. calcoaceticus* complex, suggesting the particular importance of this group.

Single copies of the *luxI* and *luxR* putative genes were detected in our samples, which is consistent with the presence of single sequences of those genes, as determined from the available sequenced genomes of *Acinetobacter*. However, the detection of several spots corresponding to AHLs upon RP-TLC separation suggested that the synthase is not specific in substrate recognition. The same feature was previously described in *A. baumannii* M2: the strain carries a single *luxI* gene copy, as confirmed with a knockout mutant, but culture supernatants display three spots on RP-TLC, each one consisting of a mixture of AHLs [21]. The high intensity of signals from *Acinetobacter pittii* RUH 0509 suggested that *Acinetobacter pittii* RUH 0509 secretes higher amounts of AHLs, or that the biosensor strain has an increased sensitivity to the AHL secreted by this strain, or a combination thereof. In this study, RP-TLC was used for AHLs detection instead of normal silica plates [12]. Although the former

slightly impairs the sensitivity of the assay, decreasing in most cases the number of spots, it increases sample resolution.

The analysis of *luxI-R* genes from *Acinetobacter* revealed high sequence identity for *luxI* and *luxR* (>85%), suggesting that the two genes are organized in tandem and co-evolved along with the Gammaproteobacteria class. This is consistent with previous reports of divergent transcription and coordinated regulation [10], but it is contrary to what was described in *Aeromonas*, in which scattered identity was determined in *luxI* and *luxR* sequences [14]. Besides, neighbor-joining trees from LuxI and from LuxR indicated that *Pseudomonas* is the most closely related genus to the *Acinetobacter* cluster. Our results are in agreement with the hypothesis that bacteria acquired their QS regulators by horizontal gene transfer in a pre-speciation stage. In fact, the overall congruity between QS and the 16SrRNA tree supports an ancient origin for QS proteins within the Gammaproteobacteria class. Note that, through horizontal gene transfer, bacteria acquire not only new structural genes but also new regulatory genes [13].

In summary, this work shows that strains of the *A. calcoaceticus-A. baumannii* complex produce specific QS signals and that their motility is inhibited by blue light, two traits that had not been previously described in this group. Additional studies are needed to determine whether there is an interaction between the two signaling systems.

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Competing interests. None declared.

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