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INQ-1, a chromosome-encoded AmpC β-lactamase from *Inquilinus limosus*

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Sir,

Since *Inquilinus limosus* was characterized in 2002,[1] infections and colonization by this species have been increasingly reported in cystic fibrosis patients (CFPs).[2–4] A multiresistant profile to antimicrobial agents, combined with its reported mucoid phenotype,[5] may explain the microorganism's ability to persist in the airways of CFPs. Nevertheless, the mechanisms associated with antibiotic resistance in *I. limosus* have not yet been elucidated. *I. limosus* MP06 was the first reported Latin-American strain recovered from a paediatric CFP at the Children's Hospital Dr Orlando Alassia (Santa Fe, Argentina, 2006).[6] Its identity was confirmed by means of 16S rRNA analysis. In this report, we describe the genetic and biochemical characterization of INQ-1 β-lactamase, a chromosome-encoded enzyme of *I. limosus* MP06.

Although screening and susceptibility tests for *Inquilinus* have not yet been standardized, they were performed according to the general CLSI guidelines with some modifications. Incubation conditions were 35°C for 30–48 h. Interpretative criteria for *Pseudomonas aeruginosa* were applied.[7] No inhibition zones were observed around β-lactam antibiotics or β-lactam/β-lactamase inhibitor discs, except for imipenem and meropenem (50 and 40 mm, respectively). MICs were determined on Mueller–Hinton broth (Britania, Argentina) using the microdilution method, after incubation for 30 h at 35°C. MICs of β-lactams corresponded to the resistant range (see Table 1), in good agreement with the disc diffusion assay, as also did those of other antibiotics such as colistin (>1024 mg/L) and gentamicin (>8 mg/L). On the other hand, this strain was susceptible to amikacin and ciprofloxacin (2 and 1 mg/L, respectively).

No plasmids were detected in *I. limosus* MP06 by conventional plasmid DNA extraction.

A 10 kb KpnI chromosomal fragment was cloned into pK19 (pK-INQ-1a) and showed a high GC content (around 70%) along the entire fragment (EMBL accession number HG326253). By *in silico* analysis, a predicted 1083 bp ORF, named bla_{INQ-1}, presented homology with AmpC β-lactamase-coding genes from various species belonging to the class *Alphaproteobacteria* (50% amino acid identity with the class C β-lactamase of *Microvirga* sp., accession number WP_009493224). Neither *ampR* nor other LysR-type regulatory genes were detected upstream of bla_{INQ-1}. In agreement, no induction of class C β-lactamase synthesis was observed when a disc assay (D-test) was performed using 6-aminopenicillanic acid, ampicillin, cefalotin, cefoxitin, imipenem or clavulanic acid as inducers.

The predicted ORF encoded a 361 amino acid protein with an unusual start codon (GTG). Putative active-site serine (included in the SLTK motif) and other putative β-lactamase motifs (YSD loop and HTG motif) were observed, compatible with the molecular description of class C β-lactamases. Predicted genes surrounding bla_{INQ-1} appear to be involved in the metabolism of recycling products and/or synthesis of peptidoglycan.

A recombinant plasmid containing a 1.4 kb XhoI–EcoRI fragment including bla_{INQ-1} was constructed using the same vector (pK-INQ-1b). The β-lactam susceptibility profile of both *Escherichia coli* INQ-1a and INQ-1b, harbouring the recombinant plasmids pK-INQ-1a and pK-INQ-1b, respectively, displayed reduced inhibition zones for ampicillin, cefalotin and piperacillin, as well as synergy with 3-phenyl boronic acid. The MICs for *E. coli* INQ-1b are summarized in Table 1.

Crude extracts showed β-lactamase activity using nitrocefin (Oxoid, UK). INQ-1 was overproduced from *E. coli* INQ-1b and purified by conventional chromatography protocols.[8] Purified INQ-1 exhibited an apparent isoelectric point of 6.0 and an apparent mol. wt of 37 kDa, which are in good agreement with the theoretical values (6.16 and 37.6 kDa, respectively). The main kinetic parameters were determined for INQ-1 by spectrophotometric analysis (Table 1). Low K_m values were determined as K_i values (apparent K_m), using cefalotin (110 μM) as reporter substrate. Although this enzyme showed preferential hydrolysis of cefalotin compared with penicillins, catalytic efficiencies to cefalotin and benzyl penicillin were similar. The turnover rate for cefalotin was 100-fold higher than that for cefoxitin. Hydrolysis of ceftazidime, ceftriaxone and imipenem was not detected. For inactivators, the IC_{50} was calculated by competition experiments or after pre-incubation (20 min) with the inactivator, using the same reporter substrate. Inactivation by clavulanic acid was observed only after pre-incubation competitive assays (indirect $IC_{50}=56$ μM). On the other hand, 3-phenyl boronic acid showed enzymatic inactivation in both competitive and pre-incubation assays at high concentrations (direct $IC_{50}=390$ μM and indirect $IC_{50}=151$ μM).

INQ-1 seems to be a cephalosporinase compatible with β-lactamases belonging to group 1 of the functional classification scheme.[9] Although INQ-1 may not explain by itself all the observed resistance to β-lactams in the clinical isolate of *I. limosus*, it contributes to the overall increase in MICs for the INQ-1-producing *E. coli*

Table 1. MICs for *I. limosus* and the transformant *E. coli* clone and kinetic parameters of INQ-1 β-lactamase

	MIC (mg/L)			Kinetic parameters[a]		
Compound	*I. limosus* MP06	*E. coli*[b] pK19	*E. coli*[b] INQ-1b	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1}.s^{-1}$)
Benzyl penicillin	NT	NT	NT	0.05	1[c]	0.048
Ampicillin	>1024	2	16	0.03	2[c]	0.017
Cefalotin	>1024	4	32	1.07	20	0.056
Cefuroxime	>1024	2	32	<0.0001[d]	4[c]	NA
Cefoxitin	256	1	1	0.01	1[c]	0.008
Ceftazidime	128	0.25	0.25	ND	ND	NA
Ceftriaxone	256	0.06	0.13	ND	ND	NA
Imipenem	0.25	NT	NT	ND	NT	NA

NT, not tested; ND, no detectable activity; NA, not applicable.
All kinetic measurements were performed at 24°C in 10 mM sodium phosphate buffer, pH 7.0.
[a]Standard deviations were <15%.
[b]*E. coli* Top 10F′ (Invitrogen).
[c]K_i values.
[d]The hydrolysis rate was too slow to obtain an accurate k_{cat}.

clone, even if transcriptional and post-transcriptional impairments are due to the unusual start codon and high GC.

Nucleotide sequence accession number

The nucleotide sequences of the bla_{INQ-1} gene and neighbour genes of *I. limosus* MP06 have been assigned the EMBL accession number HG326253

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Transparency declarations

None to declare.

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