Whole Genome Sequence Analysis of *Burkholderia contaminans* FFH2055 Strain Reveals the Presence of Putative β-Lactamases

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Abstract

Burkholderia contaminans is a member of the *Burkholderia cepacia* complex (Bcc), a pathogen with increasing prevalence among cystic fibrosis (CF) patients and the cause of numerous outbreaks due to the use of contaminated commercial products. The antibiotic resistance determinants, particularly β-lactamases, have been poorly studied in this species. In this work, we explored the whole genome sequence (WGS) of a *B. contaminans* isolate (FFH 2055) and detected four putative β-lactamase-encoding genes. In general, these genes have more than 93% identity with β-lactamase genes found in other Bcc species. Two β-lactamases, a class A (Pen-like, suggested name PenO) and a class D (OXA-like), were further analyzed and characterized. Amino acid sequence comparison showed that Pen-like has 82% and 67% identity with *B. multivorans* PenA and *B. pseudomallei* PenI, respectively, while OXA-like displayed strong homology with class D enzymes within the Bcc, but only 22–44% identity with available structures from the OXA family. PCR reactions designed to study the presence of these two genes revealed a heterogeneous distribution among clinical and industrial *B. contaminans* isolates. Lastly, bla_{PenO} gene was cloned and expressed into *E. coli* to investigate the antibiotic resistance profile and confers an extended-spectrum β-lactamase (ESBL) phenotype. These results provide insight into the presence of β-lactamases in *B. contaminans*, suggesting they play a role in antibiotic resistance of these bacteria.

Introduction

Cystic fibrosis (CF) is a genetic disorder that affects approximately 70,000–100,000 people worldwide, imposing a life-limiting condition to patients harboring this disorder [1].

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In persons with CF, *Pseudomonas aeruginosa* is one of the most important pathogens that can cause life-threatening infections. Regrettably, many other bacterial genera have also emerged as significant pathogens in persons with CF [2]. Moreover, prolonged antibiotic therapy received by these patients is a propitious scenario for the evolution of antibiotic resistant pathogens [3].

The *Burkholderia cepacia* complex (Bcc) is composed of >20 closely related species that are opportunistic human pathogens that mainly have a significant impact in patients with CF [4]. The Bcc complex is resistant to a wide range of antibiotics, and therefore infections caused by these bacteria are very difficult to eradicate. Strikingly, infections may derive into "cepacia syndrome" with fatal consequences (necrotizing pneumonia, respiratory failure, and sepsis/bacteremia) [5–7]. An additional negative aspect of the Bcc is their ability to contaminate man-made products such as pharmaceuticals, cosmetics, and disinfectants [4, 8, 9]. Nosocomial outbreaks due to the use of Bcc contaminated products are often reported [10].

Since *B. cenocepacia* and *B. multivorans* are the most prevalent Bcc among persons with CF in the United States, Canada, France, United Kingdom, and Belgium, these Bcc species are well studied and characterized [11–13]. However, in recent years, *B. contaminans*, a species defined in 2009, has been widely identified as a nosocomial opportunistic pathogen of rising importance due to outbreaks that took place in different parts of the world [14]. Presently, *B. contaminans* is reported as the most frequent Bcc species isolated in CF patients from Portugal, Spain, and Argentina, and is associated with infections in immunocompromised and hospitalized patients exposed to contaminated products in Germany and United States [15–20]. Due to its recent speciation, few studies are available on *B. contaminans*, and little is known about its virulence factors, susceptibility to antibiotics, prognosis of infected patients, and other issues of clinical relevance [21]. Recently, whole genome sequencing was performed on several *B. contaminans* strains and these sequences are available in the GenBank database [22–24].

As with all Bcc members, *B. contaminans* possesses innate resistance to a wide range of antimicrobials; as a result, the clinical treatment of infections is problematic. The main antibiotic resistance mechanisms described in Bcc include the unique structure of the lipopolysaccharide (LPS) in the outer membrane, the presence of efflux pumps, the degradation of antibiotics by means of enzymes such as β-lactamases, and the ability to form biofilms [25]. Adding to this, Bcc displays the possibility to acquire even more antibiotic resistant determinants through horizontal gene transfer [26, 27].

Among the antibiotic resistance mechanisms mentioned above, β-lactamases are not well characterized in Bcc. Inducible chromosomal class A β-lactamases (PenA and PenB) were investigated in *B. cenocepacia* and *B. multivorans* and are reported in other Bcc members [28–32]. Most studies focused on studying this single class A β-lactamase, as it appears to be the dominant β-lactam resistance mechanism in Bcc. However, the genomes of Bcc harbor other putative β-lactamase (*bla*) genes. For instance, in the study describing the whole genome of *B. cenocepacia* J2315, at least four *bla* genes encoding potential β-lactamases were found though their activity is not yet established [33]. With regard to *B. contaminans*, studies on β-lactamases have not been conducted. The presence of genes associated with β-lactamases is mentioned in a work describing the genome of *B. contaminans* MS14, an isolate obtained from soil, but the number of genes and the characterization of these enzymes was not pursued [22].

In this work, we explore the genome of the *B. contaminans* strain FFH 2055 that was isolated from the sputum of a person with CF in Argentina [34]. We determined the presence of four putative β-lactamase genes in *B. contaminans* strain FFH 2055 and conducted comparative analyses of the *bla* genes with other Bcc and non-Bcc species. The presence of select *bla* genes was further explored within 46 *B. contaminans* isolates. In addition, molecular models were generated of the class D OXA β-lactamase as well as the class A Pen-like β-lactamase, which based on previous nomenclature in the Bcc field, will be denoted as PenO [35]. As the Pen-like β-lactamases are the dominant β-lactam resistance mechanism in *Burkholderia* spp., the antibiotic resistance profile of PenO was further evaluated by cloning and expressing *bla*$_{PenO}$ in *Escherichia coli*.

Materials and Methods

Bacterial Strain and Genome Sequences

Burkholderia contaminans FFH 2055 was isolated from sputum of a 6-year-old girl with CF in Argentina. The patient was chronically infected with this species until her death at the age of 12. The strain was preserved at −80 °C in Tryptic Soy Broth (TSB) with 40% glycerol for future studies. The draft genome of FFH 2055 was recently published [34]. Sequences were retrieved from GenBank (accession LASC01000001 to LASC01000008) and analyzed to search for putative *bla* genes using the RAST server [36, 37]. Also, 46 *B. contaminans* isolates obtained from persons with CF ($n = 21$), non-CF patient comparators ($n = 13$), and industrial contaminated products ($n = 12$) were employed to investigate the distribution of *bla*$_{PenO}$ and *bla*$_{OXA-like}$ genes (Table 1).

Table 1 Putative β-lactamases in *B. contaminans* FFH2055 and % identity to non-Bcc and Bcc species

Putative β-lactamase genes in *B. contaminans* FFH2055 Locus tag (bp length)	Amino acid comparison in GenBank Best match % of identity accession product	Nucleotide comparison in *Burkholderia.com* % identity Locus tag		
		Best match	Identity with *B. cenocepacia* J2315	Identity with *B. multivorans* ATCC 17676
Class A β-lactamase WR30_RS27340 (939)	*Jeongeupia* sp. USM3 65% WP_070527757.1 Class A β-lactamase	*B. cepacia* MSMB1339WGS MSMB1339WGS_28 93.31% WK01_RS09215	89.53% BCAM2165	82.44% Bmul_3689
Class B β-lactamase WR30_RS21040 (1014)	*Amantichitinum ursilacus* 55% WP_053936944.1 MBL fold metallo-hydrolase	*B. vietnamiensis* FL-7-710-S2-D0 79.70% WJ18_RS07505	72.16% BCAS0557	–
Class C β-lactamase WR30_RS20060 (1170)	*Vogesella* sp. LIG4 63% WP_088965050.1 Class C β-lactamase	*B. lata* sp. 383 96.83% BCEP18194_RS04915	89.64% BCAS0156	81.89% Bmul_6008
Class D β-lactamase WR30_RS31065 (810)	*Klebsiella michiganensis* 61% WP_065366333.1 class D β-lactamase	*B. cepacia* MSMB2211WGS_28 94.69% WL58_RS12165	87.79% BCAM0393	–

Bioinformatics Analysis

The nucleotide and translated amino acid sequences of the putative β-lactamase-encoding genes in *B. contaminans* FFH 2055 were investigated in genome sequences of other *B. contaminans* strains as well as other Bcc species by performing BLAST N searches in GenBank and the *Burkholderia.com* database [38]. A gene encoding a Pen-like class A β-lactamase (WR30_RS27340, named *bla*$_{PenO}$) was selected for further studies. Translated amino acid sequences were analyzed using NCBI BLAST X 2.7.0+ [39]. Sequence and structural alignments of amino acid sequences were performed using T-Coffee Expresso and ESPript/ENDscript [40, 41].

Protein Structure Prediction and Molecular Modeling of Hypothetical Pen-Like and OXA-Like β-Lactamases

A prediction of the α-helical content of PenO was performed with AGADIR software [42], and theoretical three-dimensional (3D) models for the enzyme were predicted by using the Swiss-Model tool (http://swissmodel.expasy.org) and Yasara, using a standard homology modeling protocol [43]. All models were visualized with PyMOL 1.6 [44].

General Molecular Biology Techniques

The *bla*$_{PenO}$ gene was amplified by PCR reaction using primers listed in Table S1 and cloned into the pBC SK(+) vector (CHL^R) (Adgene). *E. coli* TOP10 cells were then transformed with the pBC SK(+) *bla*$_{PenO}$ (p*bla*$_{penO}$) and antibiotic susceptibility tests were performed (see below).

In addition, PCR reactions were used to detect the *bla*$_{PenO}$ and *bla*$_{OXA-like}$ genes among a collection of *B. contaminans* isolates. Primers employed in these reactions were designed using Primer 3 software (v 0.4.0) and are listed in Table S1 [45, 46]. *B. contaminans* isolates were suspended in sterile distilled water, boiled for 10 min, and centrifuged at 14000 rpm for 5 min. A 5 µl bacterial suspension was used to carry out PCR reactions. Cycling conditions were 94 °C for 2 min; 30 cycles of 94 °C for 1 min, annealing temperature for 1 min, and 72 °C for 1 min; and finally, 72 °C for 10 min.

Antimicrobial Susceptibility Testing

Disk diffusion assays with various β-lactam antibiotics (AMP: ampicillin; AMC: amoxicillin-clavulanate; CTX: cefotaxime; CRO: ceftriaxone; CAZ: ceftazidime; CZ: cefazolin; FEP: cefepime; FOX: cefoxitin; IMP: imipenem; MEM: meropenem) were performed following the Clinical and Laboratory Standards Institute (CLSI) guidelines (Table 2) [47]. The disk diffusion tests served as a screening tool to determine potential substrates for the enzyme. Minimum inhibitory concentrations (MIC) by *E*

Table 2 Origin, source, and distribution of bla_{PenO} and $bla_{OXA-like}$ genes among *B. contaminans* isolates

Isolates		Distribution of bla_{PenO} and $bla_{OXA-like}$			
Origin (number of isolates)	Source (number of isolates)	bla_{PenO}^{+} $bla_{OXA-like}^{+}$	bla_{PenO}^{-} $bla_{OXA-like}^{+}$	bla_{PenO}^{+} $bla_{OXA-like}^{-}$	bla_{PenO}^{-} $bla_{OXA-like}^{-}$
CF patients (21)	Children's Hospital Juan P. Garrahan (9)	8	0	0	1
	Children's Hospital Ricardo Gutierrez (12)	10	1	0	1
Non-CF patients (13)	University Hospital José de San Martín (5)	5	0	0	0
	Hospital Ramos Mejía (5)	4	0	1	0
	Children's Hospital Ricardo Gutierrez (3)	3	0	0	0
Industrial (12)	Purified water and facilities (5)	4	0	0	1
	Pharmaceutical products (3)	1	1	0	1
	Ultrasound gel (1)	1	0	0	0
	Disinfectants and housecleaners (3)	1	1	1	0

test for AMP, CAZ, MEM, and IMP were also performed as recommended.

Results

Putative β-Lactamases Present in the *B. contaminans* FFH 2055 Genome

The total number of putative *bla* genes in *B. contaminans* FFH 2055 genome was eighteen. However, non-β-lactamases, such as hydrolases and proteases, possess similar structures/amino acid sequences to β-lactamases; thus, proteins are often annotated as β-lactamases, even though they are not actually β-lactamases. As a result, a secondary analysis for the presence of a signal peptide as well as conserved β-lactamase motifs in translated amino acid sequences was conducted. As β-lactamases are periplasmic enzymes, they must possess a signal peptide (analysis tool: SignalP 4.1 Server). Analysis of the signal peptides in the 18 putative β-lactamases reduced the list to 5 β-lactamases (two class A enzymes, one class B, one class C, and one class D). Each β-lactamase class possesses conserved motifs that can be used to further identify potential β-lactamases. The following motifs should be present within each class: class A: SXXK motif, SDN loop, RXEXXXXXXXXXXXXD Ω-loop, and KTG motif; class B: HXHXD motif; class C: SXXK motif, YSN motif, and KTG motif; and class D: SXXK motif and KTG motif. Of the two class A β-lactamases, one lacked the SXXK, SDN, and KTG motifs, and thus was removed from the list.

As a result of this analysis, four putative *bla* genes were identified in *B. contaminans* FFH 2055. These genes encode for putative class A Pen-like (WR30_RS27340), class B (WR30_RS21040), class C (WR30_RS20060), and class D OXA (WR30_RS31065) β-lactamases (Table 1). The nucleotide sequences of WR30_RS27340 and WR30_RS20060

were found in all other *B. contaminans* genomes currently deposited in GenBank ($n = 17$) with 95–100% identity. Meanwhile, WR30_RS21040 and WR30_RS31065 were detected in one and 12 genomes (100% and 95–100% identity), respectively.

Comparative Analysis of the Four β-Lactamase Amino Acid and Nucleotide Sequences to Non-Bcc and Bcc

To further investigate these β-lactamases, a comparison to non-Burkholderial as well as Bcc β-lactamases was conducted using the GenBank and *Burkholderia.com* databases. According to GenBank, the four *B. contaminans* FHH 2055 β-lactamases were found to be most similar to bacteria (i.e., *Jeongeupia* sp. USM3, *Amantichitinum ursilacus, Vogesella* sp. LIG4, and *Klebsiella michiganensis*) isolated from environmental sources with amino acid sequence identity ranging from 55 to 65% (Table 1). A BLAST search of the *Burkholderia.com* database demonstrated that three of *bla* nucleotide sequences shared more than 93% identity with β-lactamase-encoding genes found in other Bcc species (Table 1). Only the gene coding for a putative metallo-β-lactamase (class B β-lactamase) displayed a lower identity with the closest match to a *B. vietnamiensis* strain (79.7% identity). The highest identity of individual genes was found within *B. cepacia* and *B. lata* strains (Table 1).

Furthermore, a BLAST search at amino acid level using the four putative β-lactamases detected in *B. contaminans* FFH 2055 revealed that two of them (class A and class C β-lactamase) were also found in all the Bcc species available genomes in the GenBank (only *B. arboris* does not have sequenced genomes submitted) with more than 75% and 40% of coverage and identity, respectively. The Pen-like class A β-lactamase is critical for β-lactam resistance in Bcc, while the class C β-lactamase, which was characterized in *B. multivorans,* may not actually be a β-lactamase,

but a low molecular mass penicillin binding protein; further studies are in progress (References: PMID: 30012762 and 29983287). The class B β-lactamase was not detected in *B. multivorans, B. dolosa, B. latens, B. metallica, B. seminalis, B. lata, B. metallica, B. pseudomultivorans, B. stagnalis*, and *B puraquae*. With respect to the class D β-lactamases, none were detected in *B. vietnamiensis, B. dolosa, B ambifaria*, and *B. latens* (Figure S1). Of the β-lactamases, the class A β-lactamase and class C β-lactamase are most prevalent in Bcc.

As *B. cenocepacia* and *B. multivorans* are the most prevalent species in Europe and North America, the four putative β-lactamase genes were compared to the type strain of these species (*B. cenocepacia* J2315 and *B. multivorans* ATCC 17616). The four *bla* genes found in *B. contaminans* FFH 2055 shared between 70% and 90% identity with genes of *B. cenocepacia*, while the *bla*$_{OXA-like}$ and the class B β-lactamase genes were not found in the *B. multivorans* strain (Table 1). Interestingly, the *B. cenocepacia* genes that shared similarity with *bla*$_{PenO}$ and class B β-lactamase were not annotated as β-lactamases when genome of *B. cenocepacia* J2315 was described [33].

Distribution of bla$_{PenO}$ and bla$_{OXA-like}$ Genes Among *B. contaminans* Isolates

We decided to focus our studies on two selected *bla* genes, named *bla*$_{PenO}$ and *bla*$_{OXA-like}$, coding for a class A β-lactamase and a class D β-lactamase, respectively. PenO was selected, because it is the dominant β-lactamase in Bcc. As evidence to date suggests that the class C β-lactamase may not function as a β-lactamase (PMID: 30012762), the OXA-like β-lactamase was also selected for analysis as it is next most often detected β-lactamase in Bcc.

By PCR reactions, *bla*$_{PenO}$ and *bla*$_{OXA-like}$ genes were detected in most of the *B. contaminans* isolates evaluated (84.8% *bla*$_{PenO}$ positive reactions and 87% *bla*$_{OXA-like}$ positive reactions). Slight differences were observed considering the origin of the isolates. Both PCR reactions yielded an amplification product in 37 out of 46 isolates (18 from 21 CF isolates, 12 from 13 clinical non-CF isolates, 7 from 12 industrial isolates), while in two isolates collected from CF patients and two clinical isolates, both PCR reactions resulted in negative results (Table 2). The highest percentage of isolates lacking one of these genes was obtained among industrial isolates (33.3% *bla*$_{PenO}$ and 25% *bla*$_{OXA-like}$, respectively). On the other hand, the clinical isolates obtained from non-CF patients accounted the highest percentage of positive amplification for *bla*$_{PenO}$ and *bla*$_{OXA-like}$, with only one isolate displaying a negative amplification result for *bla*$_{OXA-like}$. We note that sequence heterogeneity within the *bla*$_{PenO}$ and *bla*$_{OXA-like}$ genes may have limited the detection of some of these *bla* genes in the isolates [35].

Genetic Environment of bla$_{PenO}$ and bla$_{OXA-like}$

The surrounding of *bla*$_{PenO}$ gene displayed homologous sequences from the environment of *bla*$_{penB}$ previously described in *B. cenocepacia* (Figure S2) [29]. A gene encoding a putative LysR regulator (PenR) (WR30_RS27345) is located upstream and is transcribed in an opposite orientation of *bla*$_{PenO}$. The nucleotide sequence of this gene shares more than 94% identity with LysR encoding genes detected in *B. lata, B. cepacia*, and *B. cenocepacia* strains among other Bcc species. The 127-bp intergenic sequence between *bla*$_{PenO}$ and *penR* gene also revealed 85% similarity with the intergenic region of *B. cenocepacia* J2315 displaying identical sequences for the two putative −10 and −35 promoter boxes and the two potential ribosome-binding sites described (Fig. S2) [29].

Regarding *bla*$_{OXA-like}$ genetic environment, it is closely related to strains of other Bcc species like *B. cepacia, B. cenocepacia*, and *B. multivorans*, flanked by a putative acyltransferase and a putative porin gene (Fig. S3). Although previous described promoters were not detected in the upstream intergenic region, a BLAST comparison of this 327 bp did not reveal any matches with non-*Burkholderia* species, but shares more than 80% identity with sequences of *B. cenocepacia, B. cepacia*, and *B. lata* strains among other Bcc species, located upstream of a putative class D β-lactamase gene (Fig. S3). In addition, insertion sequences, transposons, or other mobile elements were not detected around *bla*$_{PenO}$ and *bla*$_{OXA-like}$ (data not shown).

Amino Acid Sequence Analysis and Molecular Modeling of PenO and OXA-Like β-Lactamases

Compared to other class A β-lactamases, *B. contaminans* PenO enzyme possesses all the conserved residues and motifs that model the active site (Fig. 1a): motif 1 containing the active-site serine (S70) residue (STFK); motif 2 (SDN); motif 3 (KTG); and a Ω-loop including the essential glutamic acid residue (E166). According to the amino acid relationship with other class A enzymes (Fig. 1b), *B. contaminans* PenO possesses 82% and 67% amino acid identity with *B. multivorans* PenA and *B. pseudomallei* PenI enzymes, respectively [30], and whose crystallographic structures were also solved (PDB 3W4Q and 3W4O). Regarding other Pen-like β-lactamases described in Bcc members, amino acid identities ranges from 74% (*B. ubonensis* PenH) to 88% (*B. cenocepacia* PenB1). In comparison with non-Bcc *Burkholderia* species, PenO displays identities from 57% (*B. gladioli* PenN) to 63% (*B. thailandensis* PenL). PenO also showed 55–57% amino acid identity with extended-spectrum CTX-M β-lactamases, and shares some common residues with PER enzymes, essential for the active-site coordination (see below).
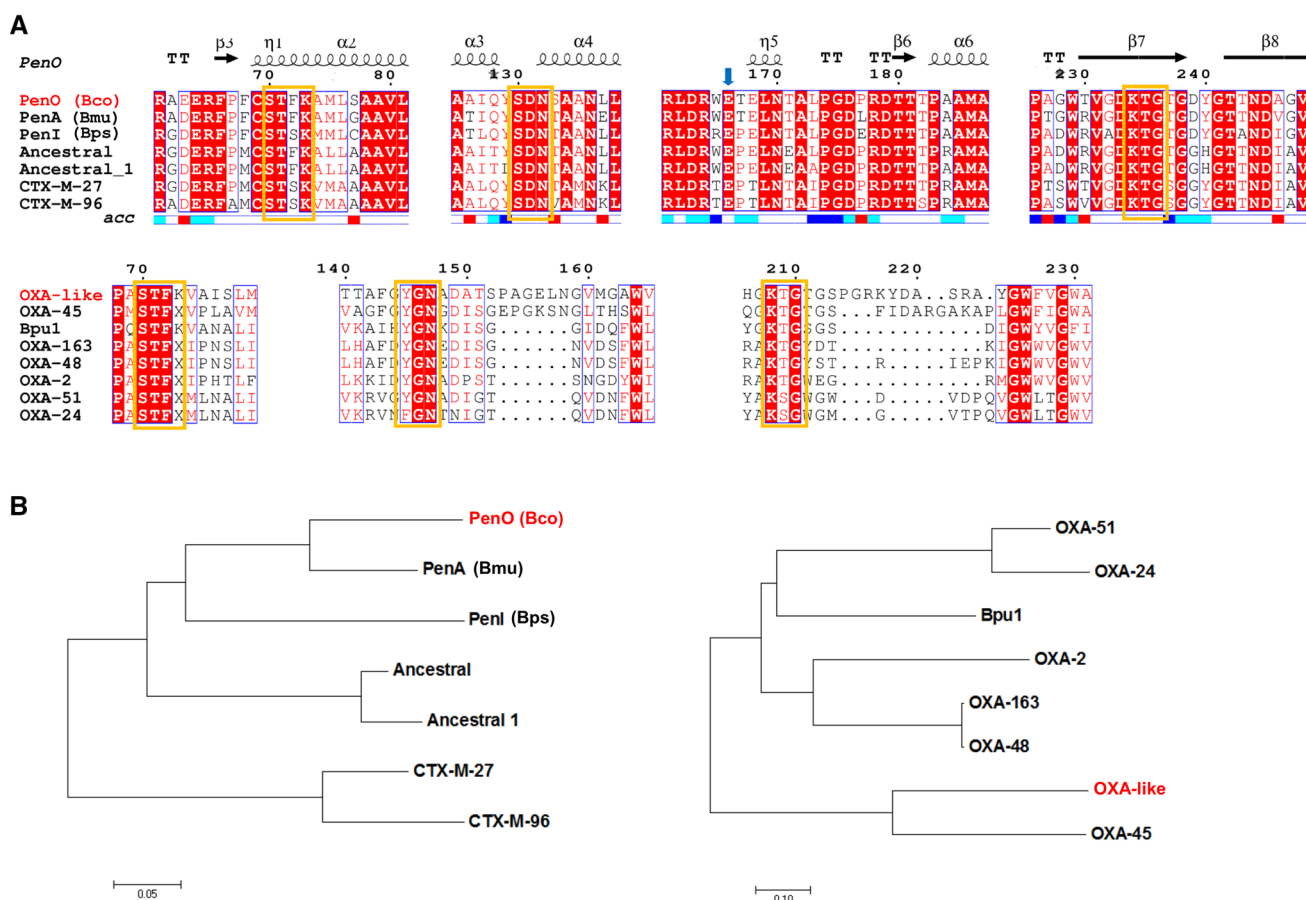
Fig. 1 **a** Multiple sequence alignment of PenO and OXA-like and other related class A and D β-lactamases, respectively, were constructed with ESPript 3.0 (http://espript.ibcp.fr/ESPript/ESPript/) and showed the regions containing the conserved active-site residues. Yellow boxes indicate the putative conserved motifs. Secondary structure is provided only for PenO, which possesses high homology with the reference sequence/model. **b** Neighbor-joining-based tree showing the amino acid sequence relationship of both enzymes with the closest β-lactamases. Sequences included were selected from the closest enzymes according to the Blast result. For those enzymes which are species-specific, the species name is provided in parenthesis: Bco: *B. contaminans*; Bmu: *B. multivorans*; Bps: *B. pseudomallei*. (Color figure online)

On the other hand, *B. contaminans* OXA-like β-lactamase showed the class D conserved motifs (Fig. 1a): STFK (motif 1) containing the active-site serine (S70); YGN (motif 2); and KTG (motif 3). OXA-like β-lactamase displayed significant homology with class D enzymes within the Bcc, 52–55% amino acid identity with OXA-57 from *B. pseudomallei*, although only 22–44% amino acid identity with available structures from the OXA family, being closely related to OXA-45 and related enzymes (Fig. 1b).

In silico molecular modeling of both PenO and OXA-like showed that these β-lactamases possess the typical overall fold of other class A and D β-lactamases, respectively, being the active-site cavity located between the "all α" and the "α/β" domains (Fig. 2).

In PenO, the active site is composed of conserved residues that are also found in other class A β-lactamases (Fig. 2b). Among the most interesting features, the presence of R220

(also found in Pen β-lactamases) and D240 are highlighted. A conserved arginine is found in most clinically important ESBL, i.e., CTX-M (R276), TEM/SHV (R244), and PER (R220) whose guanidinium lateral chain always points towards the active site [48–50]; as in the case of PER β-lactamases, R220 could also create a hydrogen bond network essential for the activity in Pen β-lactamases [51]. An aspartic acid residue at 240 is commonly found in enzymes like the cefotaxime-hydrolyzing CTX-M, which is replaced by a glycine in those variants having a slight "ceftazidimase" behavior [52, 53]. Interestingly, the putative Ω-loop fold seems to create a broader active-site cavity compared to extended-spectrum CTX-M β-lactamases, which together with the residues mentioned above could contribute to an efficient hydrolysis towards some β-lactams. Noteworthy is the presence of a threonine at 167 (also observed in PenA) replacing the conserved proline in
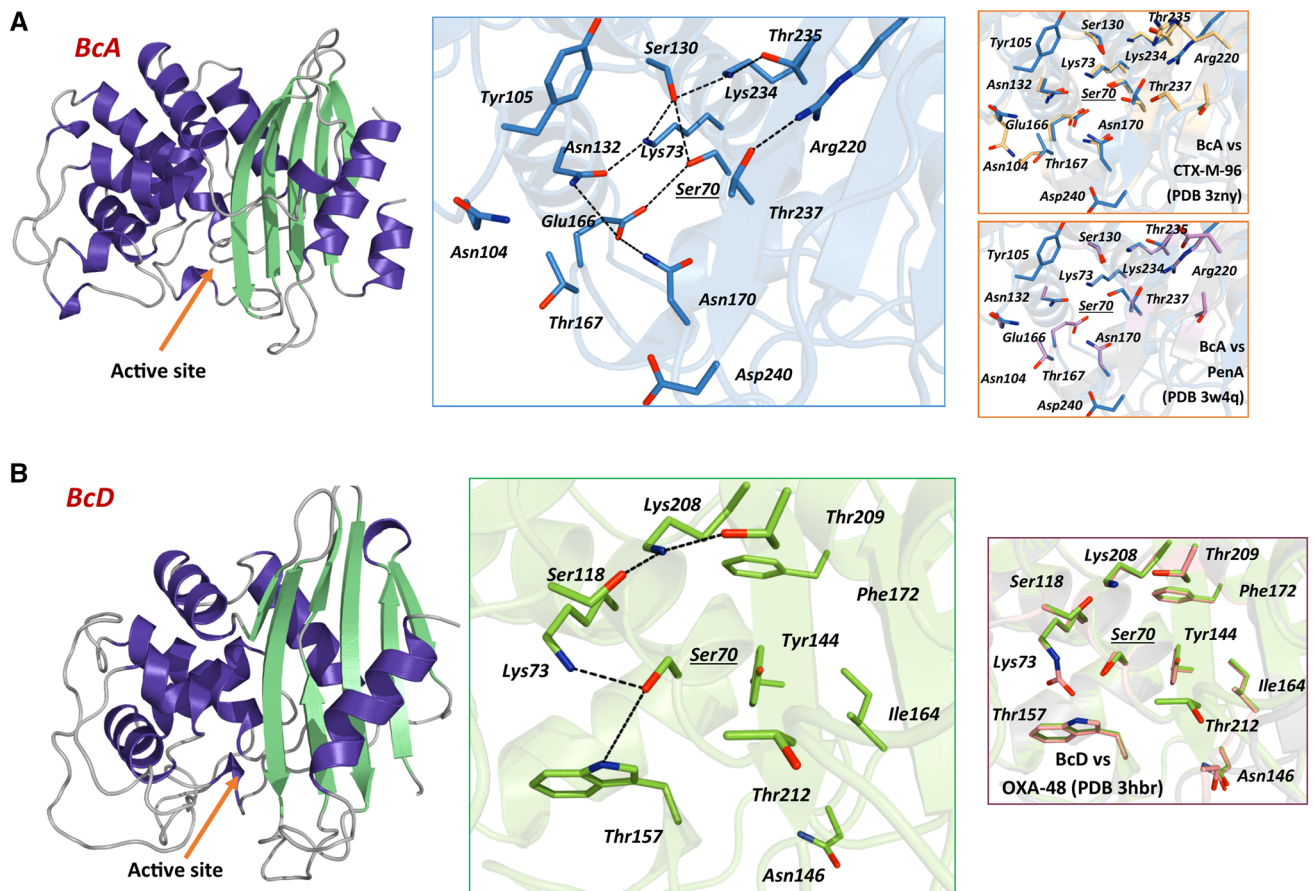
Fig. 2 Left: theoretical molecular model of PenO (up) and OXA-like (down) β-lactamases, indicating the active-site entry (orange arrow); Middle: detail of the active-site coordination, indicating conserved or important residues for the structure and function of class A β-lactamases; right: comparison of PenO vs CTX-M-96 (PDB 3ZNY) and PenA (PDB 3W4Q), and OXA-like vs OXA-48 (PDB 3HBR). (Color figure online)

most class A β-lactamases, which could influence the folding of the Ω-loop and probably also in the activity.

Remarkably, the OXA-like β-lactamase includes interesting structural features that deserve further analysis. Apart from the conserved residues usually found in class D enzymes (Figs. 1, 2), OXA-like β-lactamase remarkably carries a six-residue insertion after the YGN conserved motif (PAGELN), which is also found in OXA-45 (EPGKSN) [54]. As in other clinically important OXAs, including OXA-48-derived carbapenemases, OXA-like β-lactamase presents several mutations in the β4–β5 loop [55]; as in OXA-45, this "hot spot" region is composed of ten residues compared to five in OXA-48, OXA-24, and OXA-51 carbapenemases, while in OXA-2 and OXA-163 there is only one residue (data not shown).

Antimicrobial Phenotypic Profile of PenO β-Lactamase

As the Pen-like β-lactamases are present in all Bcc, the antimicrobial resistance profile of PenO β-lactamase was determined. Disk diffusion assays using several β-lactam antibiotics against the parent *B. contaminans* FHH 2055 and *E. coli* TOP10 cells containing plasmid, p*bla*PenO, were determined according to CLSI guidelines [47]. CLSI only provides breakpoints for ceftazidime, meropenem, and ticarcillin-clavulanic acid for Bcc. Thus, β-lactam breakpoints for Enterobacteriaceae were used to assist interpretation of the resistance profiles.

The *B. contaminans* strain demonstrated resistance towards ampicillin, amoxicillin-clavulanic acid, cefazolin, cefoxitin, cefotaxime, and ceftriaxone (Table 3). Compared to *E. coli* TOP10 harboring the empty vector, changes in the inhibition zones were observed for ampicillin, amoxicillin-clavulanic acid, cefazolin, cefoxitin, cefotaxime, ceftriaxone, and cefepime, when p*bla*PenO plasmid was introduced (Table 3). Thus, PenO behaves similarly to an extended-spectrum β-lactamase (ESBL). MIC results were also obtained for the *E. coli* strains and *E. coli* with p*bla*PenO possessed higher MICs towards all the β-lactams tested with the exception of carbapenems (Table 4).

Table 3 Average inhibition zones (in mm) obtained by disk diffusion tests from *E. coli* TOP10 derived recombinant cells producing PenO β-lactamase

	AMP	AMC	CTX	CRO	CAZ	CZ	FEP	FOX	IMP	MEM
B. contaminans FFH 2055	6	6	17	12	26	6	20	6	17	24
E. coli TOP10 pBC SK+	21	22	36	34	34	25	35	25	30	35
E. coli TOP10—p*bla*$_{PenO}$	6	12	20	10	26	6	25	21	30	35

Average disk diffusion values were determined from three independent trials

AMP ampicillin, *AMC* amoxicillin-clavulanate, *CTX* cefotaxime, *CRO* ceftriaxone, *CAZ* ceftazidime, *CZ* cefazolin, *FEP* cefepime, *FOX* cefoxitin, *IMP* imipenem, *MEM* meropenem

Table 4 Minimum inhibitory concentration (MIC) values (μg/ml) for *E. coli* TOP10 derived recombinant cells producing PenO β-lactamase

MIC	AMP	AMC	CTX	CRO	CAZ	CZ	FEP	MEM	IMP
E. coli TOP10 pBCSK+	4	2	0.047	0.047	0.38	3	0.047	0.032	0.5
E. coli TOP10—p*bla*$_{PenO}$	>256	16	2	6	2	>256	0.75	0.032	0.5

Conclusion

Although class A β-lactamases of the Pen family were reported in Bcc species and PenA in *B. multivorans* was studied and characterized, the knowledge about other β-lactamases in the Bcc is scarce [31, 55]. Particularly, studies are not available about these enzymes in *B. contaminans*, an emerging opportunistic pathogen that is also frequently recovered from contaminated products. The analysis of *B. contaminans* FFH 2055 revealed the presence of at least four putative genes coding different classes of these enzymes. A comparative analysis with other Bcc genomes demonstrated the presence, although not homogeneously, of a similar number of putative β-lactamases genes in other Bcc species. A deeper study of two of these genes, *bla*$_{PenO}$ and *bla*$_{OXA-like}$, allowed us to report the relationship with other β-lactamases, the hypothetical structure of its products, and to hypothesize that a heterogeneous distribution of these genes may occur among *B. contaminans* isolates. According to nomenclature of Pen-like family described in the *Burkholderia* genus, the name of PenO is suggested for class A β-lactamase detected in *B. contaminans*. The antibiotic susceptibility testing suggested that PenO is likely an ESBL.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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