Genetic and Biochemical Characterization of OXA-63, a New Class D β -Lactamase from *Brachyspira pilosicoli* BM4442[†]

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Brachyspira pilosicoli BM4442, isolated from the feces of a patient with diarrhea at the Hospital Saint-Michel in Paris, was resistant to oxacillin (MIC > 256 $\mu\text{g/ml}$) but remained susceptible to cephalosporins and to the combination of amoxicillin and clavulanic acid. Cloning and sequencing of the corresponding resistance determinant revealed a coding sequence of 807 bp encoding a new class D β -lactamase named OXA-63. The *bla*_{OXA-63} gene was chromosomally located and not part of a transposon or of an integron. OXA-63 shared 54% identity with FUS-1 (OXA-85), an oxacillinase from *Fusobacterium nucleatum* subsp. *polymorphum*, and 25 to 44% identity with other class D β -lactamases (DBLs) and contained all the conserved structural motifs of DBLs. *Escherichia coli* carrying the *bla*_{OXA-63} gene exhibited resistance to benzylpenicillin and amoxicillin but remained susceptible to amoxicillin in combination with clavulanic acid. Mature OXA-63 consisted of a 31.5-kDa polypeptide and appeared to be dimeric. Kinetic analysis revealed that OXA-63 exhibited a narrow substrate profile, hydrolyzing oxacillin, benzylpenicillin, and ampicillin with catalytic efficiencies of 980, 250, and 150 $\text{mM}^{-1} \text{s}^{-1}$, respectively. The enzyme did not apparently interact with oxyimino-cephalosporins, imipenem, or aztreonam. Unlike FUS-1 and other DBLs, OXA-63 was strongly inhibited by clavulanic acid (50% inhibitory concentration [IC_{50}] of 2 μM) and tazobactam (IC_{50} of 0.16 μM) and exhibited low susceptibility to NaCl (IC_{50} of >2 M). OXA-63 is the first DBL described for the anaerobic spirochete *B. pilosicoli*.

The anaerobic spirochete *Brachyspira* (previously *Serpulina*) *pilosicoli* colonizes the large intestines of pigs and of numerous other farmed and wild animals (18). In humans, *B. pilosicoli* is a cause of intestinal spirochetosis, a condition characterized by end-on attachment of the organism to colorectal epithelial cells causing abdominal pain, rectal bleeding, and diarrhea (10, 12). The therapeutic responses of patients with *B. pilosicoli* infections, the in vitro sensitivities of *B. pilosicoli* to β -lactams, and the demonstration of penicillin-binding proteins by labeling intact organisms suggest that the outer membrane is permeable to β -lactam antibiotics (6). Conversely, the resistance of *B. pilosicoli* to amoxicillin and the restoration of susceptibility by the addition of clavulanic acid (2) indicate that β -lactamase activity, rather than the inability of the drug to penetrate the outer membrane, is the predominant resistance mechanism in this spirochete.

The oxacillin-hydrolyzing β -lactamases (oxacillinases) belong to class D of the Ambler structural classification of β -lactamases (3). These enzymes form a heterogeneous group with respect to their structural and biochemical properties and share a number of unusual features: mainly, they hydrolyze oxacillin more efficiently than benzylpenicillin. In addition, they hydrolyze amoxicillin, methicillin, cephaloridine, and, to some extent, cephalothin, and their activities are usually inhibited

by NaCl (13). Only a few variants in *Pseudomonas aeruginosa* (7, 14) hydrolyze extended-spectrum cephalosporins, and an increasing number are active against carbapenems (20). With a few exceptions, OXA-type β -lactamases are poorly inhibited by clavulanic acid, being classified into group 2d of the functional classification of β -lactamases (3).

Based on their primary structure, class D β -lactamases (DBLs) have three highly conserved active-site elements in common. The first element is the Ser⁷⁰-Xaa-Xaa-Lys tetrad, where Xaa represents a variable residue, containing the active-site serine (Ser⁷⁰ according to DBL numbering) (5). The second element, Ser¹¹⁸-Xaa-Val/Ile, is equivalent to the invariable Ser-Asp-Asn motif in class A and the Tyr-Ala/Ser-Asn motif in AmpC β -lactamases, whereas the Lys²¹⁶-Thr/Ser-Gly element is common to the vast majority of serine-active β -lactamases. Other conserved motifs in DBLs are the Tyr/Phe¹⁴⁴-Gly-Asn triad and the Trp²³²-Xaa-Xaa-Gly tetrad that have no analogues in either class A or AmpC β -lactamases (20).

Although class D enzymes have been reported for a wide variety of bacterial species, such β -lactamases have not yet been described for *B. pilosicoli*. This report describes the genetic and biochemical properties of a novel molecular DBL, named OXA-63, from the human spirochete *B. pilosicoli* BM4442.

(An initial report of this work was presented at the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy [6a].)

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. *B. pilosicoli* BM4442 was isolated from the feces of a patient with diarrhea at Hospital Saint-Michel in Paris, France. *B. pilosicoli* 80.10, susceptible to β -lactams, is from our laboratory col-

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lection. *B. pilosicoli* strains were grown anaerobically at 37°C for 72 to 96 h on Columbia agar supplemented with 5% horse blood (Bio-Rad, Marnes-la-Coquette, France). *Escherichia coli* JM83 was used as a recipient for cloning the *bla*_{OXA-63} gene into the pBGS18 vector. *E. coli* BL21(DE3)pLys was used with the pET28a(+) expression vector (Novagen, Madison, WI). *E. coli* strains were grown in brain heart infusion (BHI) broth or agar at 37°C.

Susceptibility testing. Antibiotic susceptibility was tested by disk diffusion on Mueller-Hinton (MH) agar according to standards of the Comité de l'Antibiogramme de la Société Française de Microbiologie (4). MICs of antimicrobial agents for *E. coli* strains were determined by Etest (AB Biodisk, Compbourg, France) on MH agar. For *B. pilosicoli*, plates containing MH agar supplemented with 5% sheep blood were incubated in an anaerobic jar at 37°C for 3 days.

DNA preparation and transformation. Isolation of total DNA was performed as described previously (17). Restriction with endonucleases was done according to the supplier's recommendations. Amplification of DNA was performed in a 9700 thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) with *Taq* (Q-BIOgene, Inc., Carlsbad, CA) or *Pfu* (Stratagene, La Jolla, CA) DNA polymerase as recommended by the manufacturers. PCR elongation times and temperatures were adjusted to the expected size of the PCR product and to the nucleotide sequence of the primers, respectively. The amplification products were purified using the QIAquick PCR purification kit (Qiagen, Inc., Chatsworth, CA). Transformation of *E. coli* JM83 was performed as described previously (17), with selection on BHI agar containing ampicillin at 50 µg/ml and kanamycin at 40 µg/ml and on chloramphenicol (20 µg/ml) and kanamycin (50 µg/ml) for *E. coli* BL21(DE3)pLys.

DNA sequence determination and protein analysis. Sequencing of inserts in the recombinant plasmids was performed with a CEQ 2000 DNA analysis system automatic sequencer (Beckman Coulter, Palo Alto, CA). The sequence was analyzed with the GCG sequence analysis software package (version 10.1; Genetics Computer Group, Madison, WI). Blast program searches were performed by using the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>). Multiple sequence alignment of the deduced peptide sequences was carried out using the program ClustalW at the European Bioinformatics Institute website (<http://www.ebi.ac.uk/>). The SignalP 3.0 server at the ExPASy proteomics server (<http://www.expasy.org>) was used for the prediction of signal peptide cleavage sites.

Pulsed-field gel electrophoresis and Southern hybridization. A chromosomal location of the oxacillinase gene was investigated in whole-cell DNA of *B. pilosicoli* BM4442 and 80.10 by the I-CeuI technique (11). After Southern transfer, DNA was hybridized with two ³²P-labeled PCR-generated probes: an 807-bp fragment and a 721-bp fragment specific for the *bla*_{OXA-63} and 16S rRNA genes, respectively.

Production and purification of OXA-63. Two deoxyoligonucleotides, oxaF (5'-GGTGGGCTCTCCCATGTCTCTAAAAAATTCTTA-3') and oxaR (5'-CTCC TCGAGTTTTAAATAAATTATGCTTT-3'), containing BsaI and XhoI restriction sites (underlined), were used to amplify the *bla*_{OXA-63} gene from pAT784 with *Pfu* polymerase. The PCR product was cloned in the pCR-Blunt vector, resequenced, and subcloned under the control of the T7 promoter in pET28a(+) previously digested with NcoI and XhoI enzymes, producing pAT852 [pET28a(+)-Ω*bla*_{OXA-63}].

The OXA-63 enzyme engineered to have a C-terminal His₆ tag enzyme was purified from *E. coli* BL21(DE3)pLys harboring plasmid pET28a(+)-Ω*bla*_{OXA-63} as follows. The strain was grown in 0.5 liters of BHI broth containing kanamycin (50 µg/ml) and chloramphenicol (20 µg/ml) at 37°C to an optical density at 600 nm of 1.0, at which point isopropyl-1-thio- β -D-galactopyranoside was added to a final concentration of 0.5 mM. After 4 h of induction at 28°C, the cells were harvested by centrifugation and used immediately or stored at 80°C. The cell pellet was resuspended (4 ml/g of wet cells) in buffer A (20 mM sodium phosphate [pH 7.4] containing 0.5 M NaCl and 20 mM imidazole) and disrupted by sonication (five times for 30 s each time at 60 W). Cellular debris was removed by centrifugation (15,000 × g for 1 h at 4°C), and the supernatant was collected, filtered (0.22 µm), and applied to a HisTrap Fast Flow column (1 ml, 1.0-ml/min flow rate; GE Healthcare, Uppsala, Sweden) previously equilibrated with buffer A. The enzyme was eluted with a linear gradient of 20 to 500 mM imidazole over 30 ml. Fractions (1 ml each) containing the recombinant OXA-63 were analyzed for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the pure fractions were pooled, dialyzed against 20 mM sodium phosphate–0.15 M NaCl (pH 7.4), and concentrated to 1 mg/ml with a Centrprep 30 concentrator (Amicon). Protein purity was estimated by SDS-PAGE analysis. The enzyme concentration was determined on the basis of *A*₂₈₀ assuming an ϵ of 35410 cm⁻¹ M⁻¹ as calculated by ProtParam of the ExPASy proteomics server. The purified enzyme (1 mg/ml) was stored at 4°C.

NH₂-terminal sequencing of OXA-63. The presence of a putative signal peptide cleavage site was investigated by N-terminal Edman sequencing on an

TABLE 1. MICs of β -lactams for *B. pilosicoli* and *E. coli* strains with or without OXA-63

β -Lactam	MIC (µg/ml) against ^a :			
	<i>B. pilosicoli</i>		<i>E. coli</i>	
	80.10	BM4442	JM83	JM83 + OXA-63
Penicillin G	0.023	2	24	>256
Amoxicillin	0.047	1.5	6	>256
Amoxicillin-clavulanate	0.047	0.25	3	12
Oxacillin	0.125	>256	NA	NA
Piperacillin	0.016	48	1.5	96
Piperacillin-tazobactam	<0.016	0.016	1	1.5
Ceftazidime	4	4	0.5	0.5
Cefotaxime	<0.016	0.032	0.064	0.064
Imipenem	<4	<4	<4	<4
Meropenem	0.032	0.032	0.032	0.016
Aztreonam	1	1	0.19	0.19

^a The susceptibilities of *B. pilosicoli* 80.10 and *E. coli* JM83 are shown for comparison. NA, not applicable; *E. coli* JM83 is naturally resistant to oxacillin.

Applied Biosystems Procise sequencer with the reagents and methods recommended by the manufacturer.

Determination of kinetic parameters of OXA-63. Purified OXA-63 was used for the determination of kinetic parameters (k_{cat} and K_m), which was performed at 25°C in 100 mM sodium phosphate (pH 7.0) in a total volume of 0.5 ml. The initial rates of hydrolysis were determined with a Uvikon UV931 spectrophotometer (Kontron Instruments). The wavelengths and changes in extinction coefficients of β -lactams were previously described (16). The enzyme concentration in the reaction mixture was in the range of 50 to 150 nM. The steady-state kinetic parameters were determined using the Hanes-Woolf plot (8). The 50% inhibitory concentration (IC₅₀) was determined as the β -lactamase inhibitor or NaCl concentrations that reduced the hydrolysis rate of 100 µM nitrocefin by 50% when the enzyme was preincubated with various concentrations of inhibitor for 30 min at 25°C before the addition of the substrate (14).

Size-exclusion chromatography. Size-exclusion chromatography was performed using a Hiload 16/60 Superdex 75 column (GE Healthcare) equilibrated with 50 mM sodium phosphate buffer containing 0.15 M NaCl (pH 7.0) and eluted in the same buffer at a flow rate of 1 ml/min. The column was calibrated using aprotinin (M_r , 6,500), RNase A (M_r , 13,700), carbonic anhydrase (M_r , 29,000), and conalbumin (M_r , 75,000).

Nucleotide sequence accession number. The nucleotide sequence of the *bla*_{OXA-63} gene and of the flanking regions has been deposited in the GenBank database under accession number AY619003.

RESULTS AND DISCUSSION

Antibiotic resistance of *B. pilosicoli* BM4442. The clinical isolate was resistant to penicillin G (MIC = 2 µg/ml), ampicillin (MIC = 1.5 µg/ml), and oxacillin (MIC > 256 µg/ml). The strain hydrolyzed nitrocefin but remained susceptible to cephalosporins and to the combination of amoxicillin and clavulanic acid (Table 1 and data not shown).

Cloning and sequencing of the DBL gene. Total DNA from BM4442 cleaved by Sau3AI was ligated into BamHI-digested pBGS18 DNA and transformed into *E. coli* JM83. Transformants selected on medium containing ampicillin plus kanamycin were screened for their plasmid contents by agarose gel electrophoresis of crude bacterial lysates. The smallest recombinant plasmid contained an 8-kb insert. Subcloning of an internal 2-kb HindIII fragment into pBGS18 generated pAT784, which conferred resistance to amoxicillin and piperacillin but not to amoxicillin in combination with clavulanate (Table 1).

Comparison of the sequence of the insert of pAT784 with

	-35	-10	RBS	
tatatagcagttgacaatcatatacaaatattaatatataactccgcaaaaatatcaaggatta				60
taaATGCTCTAAAAAATTTTATATTAATATTTATTTTGTATTTTAAATATCTGTAAA				120
M S K K N F I L I F I F V I L I S C K				19
AATACAGAAAAATATCAAATGAACTACATTAATAGATAATATATTTACTAATAGCAAT				180
N T E K I S N E T T L I D N I F T N S N				39
GCTGAAGGAACATTAGTTATATATAAATTAATGATGATAAATATATAATTCATAATAA				240
A E G T L V I Y N L N D D K Y I I H N K				59
GAAAGAGCTGAACAAAGATTTTATCCAGCATCAACATTTAAAAATATATAATAGTTTAATA				300
E R A E Q R F Y P A <u>S T F K</u> I Y N S L I		70	73	79
GGCTTAAATGAAAAAGCAGTTAAAGATGTAGATGAAGTATTTTATAAATTAATGGCGAAA				360
G L N E K A V K D V D E V F Y K L M A K				99
AGTTTCTCTGAATCTTGGGCTAAAGACTCTAATTTAAGATATGCAATAAAAATTCGCAA				420
S F L E S W A K D S N L R Y A I K N <u>S O</u>			118	119
GTACCGCATATAAAGAATTAGCAAGAAGAATAGGTATTAAGAAGATGAAAGAGAATATA				480
<u>V</u> P A Y K E L A R R I G I K K M K E N I				139
	120			
GAAAACTAGATTTTGGTAATAAAAGTATAGGTGATAGTGTAGATACTTTGGCTTGAA				540
E K L D <u>F G N</u> K S I G D S V D T F W L E		144	146	159
GGACCTTTGGAATAAGTGGGATGGAGCAAGTTAAATTATTAATAAATTAGCTCAAAAT				600
G P L E I S A M E Q V K L L T K L A Q N				179
GAATTACAGTATCCTATAGAAATACAAAAGCTATTTCTGATATTACTATTACTAGAGCA				660
E L Q Y P I E I Q K A I S D I T I T R A				199
AACTTACATATTACGCTTCATGGAAAACTGGATTAGCTGATTCTAAAAACATGACAACT				720
N L H I T L H G <u>K T G</u> L A D S K N M T T		216	218	219
GAGCCTATTGGTTGGTTTCGTAGCTGGCTTGAAGAAAATGATAATATATACGTCTTTGCT				780
E P I G <u>W F V G</u> W L E E N D N I Y V F A		232	235	239
TTAAATATTGATAATATCAATTGACCTTGCAAAAAGGATAAATATAGTAAAAGAA				840
L N I D N I N S D D L A K R I N I V K E				259
AGTTTAAAGCATTAATTTATTAATAAAttatataaaaaattataaatgatttttaaa				900
S L K A L N L L K *				268

FIG. 1. Deduced sequence of OXA-63 aligned with the corresponding gene. The -35 (TTGACA) and -10 (TATACT) regions and the ribosome binding site (RBS) are indicated in boldface type. The motifs usually conserved among DBLs are double underlined, and their location is indicated according to the DBL numbering system (5). The star indicates the stop codon.

sequences in the GenBank database revealed 70% identity over 620 nucleotides to the *bla*_{FUS-1} gene belonging to DBLs (EMBL/GenBank accession number AY227054), and the gene was named *bla*_{OXA-63}. A putative ribosome binding site, AAGGA, was located 6 bp upstream from an ATG initiation codon leading to a sequence of 807 bp. Analysis of the upstream sequence indicated the presence of a putative promoter composed of -35 TTGACA and -10 TATACT motifs separated by 17 bp (Fig. 1).

B. pilosicoli harbors a single *rm* operon leading, after I-CeuI restriction, to a ca. 3.2-Mb DNA fragment, which remained in the plug. However, the G+C content of the *bla*_{OXA-63} gene (25%) was similar to that of the *Brachyspira* sp. chromosome. The sequence of ca. 700 bp of the flanking regions did not show any structure reminiscent of transposons or integrons. Additionally, further upstream from the *bla*_{OXA-63} gene, there was a 156-bp sequence that was 72% identical to a chromosomal sequence from *Brachyspira hyodysenteriae*. Taken together, these data indicate that the *bla*_{OXA-63} gene was chromosomally located in *B. pilosicoli* BM4442.

Structural properties of OXA-63. OXA-63 exhibited the three highly conserved active-site elements of DBLs. The first one, the Ser-Thr-Phe-Lys tetrad containing the active-site serine, was found at positions 70 to 73 (Ser⁷⁰ according to DBL numbering) (5); the second element, Ser-Gln-Val, was at DBL 118 to 120; and the third, Lys-Thr-Gly, was at positions 216 to 218. The Tyr-Gly-Asn triad (DBL positions 144 to 146) found in oxacillinases was replaced by the Phe-Gly-Asn motif as in most carbapenem-hydrolyzing oxacillinases, and the Trp-Phe-Val-Gly tetrad was at positions 232 to 235 (Fig. 1).

OXA-63 had 25 to 44% identity with various DBLs and shared 53% identity with FUS-1 (OXA-85), a recently described oxacillinase from the anaerobic gram-negative rod *Fusobacterium nucleatum* subsp. *polymorphum* (19). Phylogenetic analysis indicated that OXA-63 exhibited the closest ancestral relationship with putative products from the genomes of *Campylobacter jejuni* (OXA-61) (1) and *Campylobacter lari* RM2100 (GenBank accession no. ZP_00369367) and clustered with a group of chromosomally encoded enzymes including OXA-50 (7) and some homologues detected in the genomes of

TABLE 2. Kinetic parameters of purified OXA-63 β -lactamase for hydrolysis of some β -lactams in comparison with FUS-1^a

Substrate	k_{cat} (s ⁻¹)		K_m (μ M)		k_{cat}/K_m (M ⁻¹ s ⁻¹)		Relative k_{cat}/K_m	
	OXA-63	FUS-1	OXA-63	FUS-1	OXA-63	FUS-1	OXA-63	FUS-1
Oxacillin	113	780	115	36	9.8×10^5	2.1×10^7	100	25
Benzylpenicillin	4.9	830	19	10	2.5×10^5	8.3×10^7	25.5	100
Ampicillin	6.6	45	43	76	1.5×10^5	5.7×10^5	15	0.7
Carbenicillin	1.1	NA	17	NA	6.5×10^4	NA	6.6	NA
Piperacillin	2.1	NA	33	NA	6.4×10^4	NA	6.5	NA
Cephalothin	ND	4.1	—	47	—	8.7×10^4	—	0.1
Cefuroxime	ND	>1.8	—	>400	—	4.5×10^3	—	0.005

^a Data are the means of three independent determinations. Standard deviations were within 10% of the means. Relative values were calculated according to that obtained for oxacillin (OXA-63) and for benzylpenicillin (FUS-1), which were set at 100. Kinetic data for FUS-1 (OXA-85) are from reference 19. NA, not available; ND, no detectable hydrolysis; —, not determinable.

various bacterial species such as *Thiomicrospira denitrificans* ATCC 33889 (accession no. YP_393464) and *Methylobacillus flagellatus* KT (accession no. ZP_00564423).

Purification and characterization of the OXA-63 β -lactamase. The carboxy-terminal His₆-tagged OXA-63 was expressed in *E. coli* BL21(DE3)pLys harboring plasmid pAT852 [pET28a(+) Ω bla_{OXA-63}]. The weak rate of expression (ca. 5% of total proteins) was possibly due to the low G+C content (25%) of the bla_{OXA-63} gene. OXA-63 was purified from the crude lysate by single-step nickel affinity chromatography. The protein was >95% pure, as judged by SDS-PAGE analysis (data not shown). Overall, the procedure yielded approximately 2 mg of purified OXA-63 per liter of culture. The M_r of the native protein determined by size-exclusion chromatography was estimated to be 63 kDa, suggesting that the native protein is a dimer.

The SignalP 3.0 server suggested that OXA-63 could be a nonsecreted protein. This was confirmed by the N-terminal amino acid sequencing of the mature protein, which did not reveal a cleavage site. The SKKNFI sequence obtained corresponded to that of the native protein without the methionine. Moreover, study of the location of OXA-63 in *E. coli* by Western blotting indicated that the majority of the enzyme was present in the membrane fraction, with only small amounts in the cytoplasmic and periplasmic fractions. These data suggest that OXA-63 could be the first example of a membrane-located β -lactamase in gram-negative bacteria.

Kinetic parameters indicated that the OXA-63 β -lactamase has a narrow substrate profile that includes benzylpenicillin, oxacillin, and ampicillin (Table 2). Oxacillin was the best substrate, with a high turnover rate (k_{cat} , 113 s⁻¹) and consequently a high catalytic efficiency (k_{cat}/K_m , 9.8×10^5 M⁻¹ s⁻¹), being hydrolyzed four- and sixfold more efficiently than benzylpenicillin and ampicillin, respectively. Despite the high affinity (low K_m values) of OXA-63 for ampicillin, benzylpenicillin, piperacillin, and carbenicillin, the turnover rates were low (k_{cat} of <10 s⁻¹). Even at high concentrations (1 μ M), OXA-63 did not hydrolyze cefotaxime, ceftazidime, cefepime, imipenem, and aztreonam. Interestingly, OXA-63 had no carbapenem-hydrolyzing activity, although it had the Phe-Gly-Asn triad present in most carbapenem-hydrolyzing oxacillinases. This is in agreement with mutagenesis studies showing that the Phe residue at DBL position 144 is not, by itself, responsible for carbapenem-hydrolyzing activity (9, 15). No biphasic kinetic was observed for all the substrates tested.

Compared to the closely related FUS-1 (OXA-85), OXA-63 exhibited differences in kinetic parameters resulting in variations in the substrate spectra of activity. The catalytic efficiencies, k_{cat}/K_m , for penicillins of FUS-1 were much higher than those of OXA-63. With oxacillin, a higher K_m value was obtained, with OXA-63 resulting in k_{cat}/K_m ratios that were 20-fold lower than those measured with FUS-1. Unlike FUS-1, cephalothin and cefuroxime were not hydrolyzed by OXA-63 even at high enzyme concentrations (1 μ M).

In contrast with FUS-1 and other DBLs, OXA-63 was strongly inhibited by clavulanate (IC₅₀, 2 μ M), tazobactam (IC₅₀, 0.16 μ M), and sulbactam (IC₅₀, 12 μ M). These data are in agreement with the susceptibility patterns of *B. pilosicoli* BM4442 and *E. coli* JM83 carrying the cloned bla_{OXA-63} gene. The strains were resistant to most penicillins, whose antimicrobial activities were restored by clavulanic acid and tazobactam (Table 1). OXA-63 was poorly inhibited by NaCl (IC₅₀, >2 M), which represents an important difference from other oxacillinases that are usually inhibited by chloride ions (IC₅₀, 250 mM) (13). Resistance to inhibition and, conversely, susceptibility to inhibition by NaCl are related to the presence of Phe and Tyr, respectively, at DBL position 144 (13, 15). OXA-63 was resistant to NaCl (IC₅₀, >2 M), suggesting that the Phe¹⁴⁴ residue may prevent NaCl from affecting the active site.

In conclusion, this work characterizes the genetics and biochemistry of the new narrow-spectrum OXA-63 DBL from *B. pilosicoli* BM4442.

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