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SUSCEPTIBILITY

Tn*6350*, a Novel Transposon Carrying Pyocin S8 Genes Encoding a Bacteriocin with Activity against Carbapenemase-Producing *Pseudomonas aeruginosa*

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ABSTRACT A novel transposon belonging to the Tn*3*-like family was identified on the chromosome of a commensal strain of *Pseudomonas aeruginosa* sequence type 2343 (ET02). Tn*6350* is 7,367 bp long and harbors eight open reading frames (ORFs), an ATPase (IS*481* family), a transposase (DDE catalytic type), a Tn*3* resolvase, three hypothetical proteins, and genes encoding the new pyocin S8 with its immunity protein. We show that pyocin S8 displays activity against carbapenemase-producing *P. aeruginosa*, including IMP-1, SPM-1, VIM-1, GES-5, and KPC-2 producers.

KEYWORDS transposon, S-type pyocins, bacteriocin, *Pseudomonas aeruginosa*, metallo-β-lactamases

Pseudomonas aeruginosa is an opportunistic pathogen and a leading cause of nosocomial infections, which are very difficult to treat because hospital-associated lineages are usually multidrug resistant (MDR). Carbapenems have been considered the most effective drugs against MDR isolates. However, the emergence and dissemination of carbapenemase-producing *P. aeruginosa* strains have become a major health care problem (1, 2). Therefore, since therapeutic options are limited, the research of new therapeutic compounds is essential. In this regard, one alternative strategy has been the investigation of natural antibiotics (i.e., bacteriocins) produced by many bacteria for intraspecies competition (3–9). Specifically, bacteriocins produced by *P. aeruginosa* are called "pyocins." Based on their structure, pyocins can be divided into three types, named R, F, and S. While R- and F-type pyocins are high-molecular-weight protein complexes that resemble phage tails, S-type pyocins are binary protein complexes consisting of a large protein that harbors the killing function and a smaller immunity protein that confers protection against the cognate bacteriocin (9, 10). Most S-type pyocins bind to specific receptors in the bacterial outer membrane and are translocated through it before killing their target. In *P. aeruginosa*, several S-type pyocins exhibiting different killing activities, such as DNase (S1, S2, S3, and AP41), tRNase (S4, S11, and S12), pore-forming (S5), rRNase (S6), and lipid II degradation activity (M1 and M4), have been described and functionally characterized. Currently, genes encoding S-type pyocins S7 through S10 have been identified *in silico* by analysis and comparison of draft and complete genome sequences of *P. aeruginosa* strains (9).

In this study, we performed a screening for strains producing pyocins with activity against MDR *P. aeruginosa* strains. In this regard, pyocins extracted from a commensal strain (ET02), belonging to sequence type ST2343, which was isolated from a healthy

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TABLE 1 Bacterial activity of pyocin S8 against MDR *Pseudomonas aeruginosa* strains isolated from clinical and environmental samples

P. aeruginosa strain	Source	Resistance profile for[a]:								β-Lactamase[b]	Bacterial activity of pyocins[c]:	
		IPM	MEM	CAZ	FEP	AK	GEN	LVX	CIP		S8	S2, S4, S5[d]
48-1997A	Human	R	R	R	R	R	R	R	R	SPM-1	+	–
247-B	Human	R	R	R	I	S	R	R	R	VIM-1	+	–
GIM	Human	R	R	R	R	I	R	R	R	GIM-1	+	–
BH6	Human	R	R	R	R	R	I	R	R	KPC-2	+	–
PHB64	Human	R	R	R	R	R	R	R	R	GES-5	+	–
IMP	Human	R	R	R	R	R	R	R	I	IMP-1	+	–
298	Human	I	R	R	R	S	I	S	S	OXA-18	+	–
395	Human	R	R	R	R	R	R	R	R	IMP-18	–	–
179	Human	R	R	R	R	R	R	R	R	VIM-1	+	–
1088	Human	R	R	R	R	S	I	R	R	SPM-1	+	–
392	Human	R	R	I	R	S	R	R	R	OXA-10	–	–
225	Human	R	R	R	R	R	R	R	R	GES-1	+	–
44	Human	R	R	R	R	R	R	R	R	SPM-1	+	–
19	Environmental	R	R	R	R	R	R	R	R	SPM-1	+	–
141	Environmental	R	R	R	R	R	R	R	R	SPM-1	–	–
151	Environmental	R	R	R	R	R	R	R	R	SPM-1	+	–
ET02	Human	S	S	S	R	S	I	I	S	OXA-50	–	+
PAO1	Control	S	S	S	S	S	S	S	S	OXA-50	+	–

[a]IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; FEP, cefepime; AK, amikacin; GEN, gentamicin; LVX, levofloxacin; CIP, ciprofloxacin (21); R, resistant isolates; I, intermediate isolates; S, susceptible isolates.
[b]Genes encoding β-lactamases were confirmed by PCR and sequencing.
[c]Bacterial activity of pyocins was evaluated by the presence (+) or absence (–) of inhibition zones measuring ≥10 mm, using a pyocin assay (5).
[d]Pyocins produced by *P. aeruginosa* strain PAO1 (9).

patient in Brazil, exhibited the highest antibacterial activity against MDR *P. aeruginosa* isolates, including carbapenemase-producing nosocomial lineages (Table 1).

The antimicrobial activity of pyocins from the ET02 strain was evaluated using a previously described pyocin assay method (5). In brief, the pyocin production in ET02 was induced by adding a 3 μg/ml final concentration of mitomycin C in culture medium. Pyocin molecules were then precipitated by ammonium sulfate. The high-molecular-weight R- and F-type pyocins were sedimented by ultracentrifugation, whereas S-type pyocins were recovered from the supernatant (11). Both high- and low-molecular-weight pyocins were screened against carbapenemase-producing *P. aeruginosa* isolates, where the fraction containing the S-type pyocins from the ET02 strain displayed the highest antibacterial activity against clinically significant β-lactamase (SPM-1, GIM-1, VIM-1, IMP-1, KPC-2, OXA-18, GES-1, and GES-5)-producing *P. aeruginosa* strains (12–17) (Table 1). In contrast, S-type pyocins produced by *P. aeruginosa* PAO1 (i.e., S2, S4, S5) (9), which were used as a control, showed no activity against the strain panel (Table 1). S-type pyocins from the ET02 strain were submitted to proteomic analysis by mass spectrometry, and data were searched against the Swiss-Prot database, resulting in 19% coverage of the pyocin AP41 sequence (GenBank accession number Q51502).

Total genomic DNA of *P. aeruginosa* ET02 was extracted to construct a mate-paired library, which was sequenced using the MiSeq platform (Illumina, Inc.). The sequence reads were *de novo* assembled using an A5-miseq pipeline (18), and after automatic annotation using Prokka (www.github.com/tseemann/prokka), the sequence was manually curated using the GenBank database and InterPro (www.ebi.ac.uk/interpro). Curiously, during whole-genome sequencing (WGS) analysis, we noted that a DNA region of 7,367 bp containing the S-type pyocin nucleotide sequence from strain ET02 displayed 100% identity with a region analyzed from the complete genome sequence of *P. aeruginosa* strain BAMCPA07-48, recently submitted to the NCBI GenBank database (accession number CP015377.1). This region contained a transposon DNA-invertase, hypothetical proteins, and an uncharacterized S-type pyocin. Additionally, a translated search of GenBank (blastx) revealed that the S-type pyocin from ET02 displayed 90% identity to AP41 pyocin (GenBank accession number Q51502).

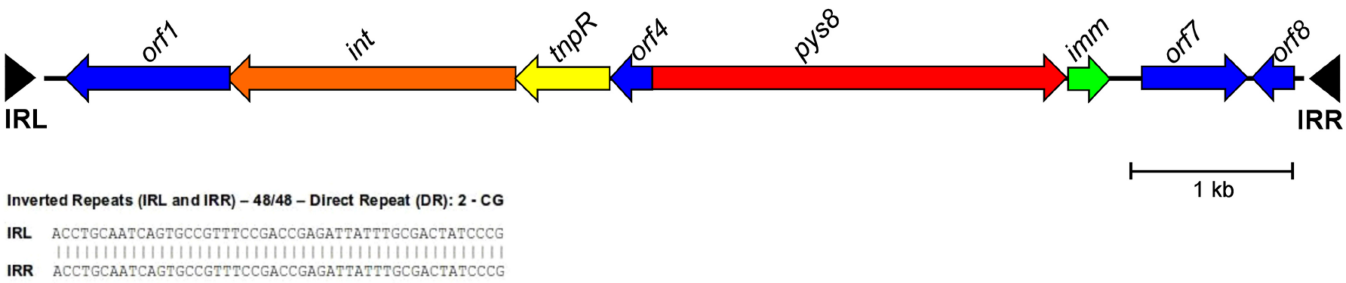

FIG 1 Genetic organization of Tn*6350* (GenBank accession number KY347015), which harbors genes encoding pyocin S8. Predicted coding regions are represented by thick arrows indicating the direction of transcription. This element contains eight ORFs, including an ATPase (*orf1*), a transposase (*int*), a resolvase (*tnpR*), a cytotoxic subunit of pyocin S8 (*pys8*), an immunity subunit of pyocin S8 (*imm*), and three more hypothetical proteins (*ofr4*, *orf7*, and *orf8*). The inverted repeat (IR) sequences flanking the element are marked by black triangles (IRL, inverted repeat left; IRR, inverted repeat right). They have 48 bp and 100% identity. The element is flanked by short direct repeats (DRs) rich in CG dinucleotide.

Primers were designed to confirm the nucleotide sequence of ET02 pyocin by Sanger sequencing, and then the translated sequence was deduced and analyzed; 772 amino acids were obtained. Although attempts to perform a comparative protein analysis using different databases were unsuccessful, we found a recent review of antibacterial proteins and peptides of *Pseudomonas* (9), in which new S-type pyocins from *P. aeruginosa* were identified based on an *in silico* analysis, where a pyocin named S8 was identical to the pyocin produced by *P. aeruginosa* ET02.

The genetic environment of S8 pyocin from ET02 was submitted to the Tn Number Registry database (http://www.ucl.ac.uk/eastman/research/departments/microbial-diseases/tn), confirming a new transposon, which was designed Tn*6350*. In this regard, Tn*6350* is a 7,367-bp transposon (GenBank accession number KY347015) belonging to the Tn*3*-like family, which was located on the chromosome of ET02. This transposon carries genes encoding eight ORFs, an ATPase (IS*481* family), a transposase (DDE catalytic type), a resolvase (Tn*3* family), an S-type pyocin, an immunity protein, and three hypothetical proteins (Fig. 1).

S-Type pyocins carried by transposon elements have been previously described (19). Transposons are genetic elements able to move within and among genomes. These elements are important modulators of bacterial genomes, in many cases improving the environmental adaptation (20). In recent years, WGS approaches have allowed the *in silico* comparative analysis of bacterial genomes. In this context, novel transposable elements have been identified. So, although Tn*6350* has not been previously described and characterized, it might be present in other *P. aeruginosa* strains.

Interestingly, pyocin S8 (harbored by Tn*6350*) exhibited a wide bactericidal activity against clinically significant carbapenemase-producing *P. aeruginosa* strains. For the host strain, this bacteriocin production can confer a fitness advantage, whereas the potency and targeted action of this pyocin could be developed into a clinically useful antibiotic against MDR pathogens for which there are few therapeutic options (4).

In summary, we hereby describe a new transposon carrying S8 pyocin genes encoding a bacteriocin presenting activity against carbapenemase-producing *P. aeruginosa* strains. The clinical therapeutic potential of this new pyocin is worthy of further investigation.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number LYLY00000000.

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We have no conflicts of interest to declare.

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