Cloning and Characterization of *A1S_0255 and A1S_3219*, Putative Outer Membrane Factors of *Acinetobacter baumannii* ATCC19606

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ABSTRACT

Acinetobacter baumannii is a multidrug resistant opportunistic pathogen found worldwide. Antibiotic resistance of this organism is usually a function of multidrug efflux pumps belonging to the Resistance-Nodulation-cell Division (RND) family. RND pump complexes consist of three components: Outer Membrane Factor (OMF), Membrane Fusion Protein (MFP) and the RND protein. Together these three proteins are capable of pumping out the antibiotic molecule into the external environment. In this study, we are cloning and characterizing two putative OMF-encoding genes, *A1S_0255* and *A1S_3219*. Artificial operons are being constructed using the cloned OMF-encoding genes and MFP and RND protein-encoding genes of *A. baumannii*. The operons will be characterized in a surrogate *Pseudomonas aeruginosa* strain in single copy. This study will provide insights into the mechanisms of multidrug resistance in *A. baumannii* which in turn will aid in designing better and more effective drug therapy.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative bacterial species that is an emerging nosocomial pathogen implicated in urinary tract infections, pneumonia and meningitis [1]. It is resistant to various antimicrobial agents and multidrug resistant (MDR) strains have been isolated worldwide [2]. Role of *A. baumannii* in war-related injuries is now well documented [3] with increasing number of soldiers serving in Iraq and Afghanistan getting infected with the MDR strains from soil. A number of factors are known to contribute to the drug resistance in *A. baumannii* but energy-dependent efflux of drugs mediated by proteins belonging to Resistance-Nodulation-Division (RND) family is now being accepted as the major mechanism of its antibiotic resistance. These proteins form a tripartite structure consisting of an outer membrane factor (OMF), RND drug transporter and a membrane fusion protein (MFP) [4] (Fig 1).

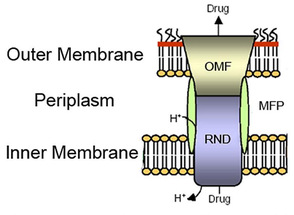


Fig. 1. Representation of RND efflux pumps [4]

We have identified a putative RND pump-encoding operon *A1S_2817-A1S_2818* in *A. baumannii*. This operon encodes for the RND pump and MFP but lacks the gene for an OMF. Using a mini-Tn*7*-based insertion system, we are characterizing *A1S_2817-A1S_2818* operon of *A. baumannii* in a surrogate *Pseudomonas aeruginosa* PAO750 that lacks native efflux pumps. We did not observe any changes in the resistance profile of the *P. aeruginosa* PAO750 expressing *A1S_2817-A1S-2818. A1S_2817-A1S_2818* was then inserted into *P. aeruginosa* PAO325 that expresses an OMF called OpmH, however the resultant strain failed to show any changes in the susceptibility profile from that of the parent strain. We have also identified two OMF-encoding genes, *A1S_0255* and *A1S_3219* in *A. baumannii* and the purpose of this study is to determine whether the gene products of these two OMF-encoding genes form a functional complex with *A1S_2817-A1S_2818* pump.

METHODS

1. Bacterial Strains

Acinetobacter baumannii strain ATCC 19606
E. coli GBE180
Pseudomonas aeruginosa PAO750
Pseudomonas aeruginosa PAO325
Pseudomonas aeruginosa PA002
Pseudomonas aeruginosa PA004

Table 1. Antibiotic susceptibilities of *Pseudomonas aeruginosa* strains expressing *A1S_2817-A1S_2818*

Antibiotics	AMP	AMC	PIP	TZP	CEF	CFZ	CXM	FOX	CFM	CPD	CTX	CAZ	IPM	AMK	GEN	TOB	CIP	TET	NIT	SXT
PA002	≥32	≥32	≤4	≤4	>64	≥64	≥64	≥64	≥4	≥8	32	≤1	≤1	≤2	≤1	≤1	≤0.25	≤1	128	≤20
PA002 (+ IPTG)	≥32	≥32	≤4	≤4	>64	≥64	≥64	≥64	≥4	≥8	≥64	≤1	≤1	≤2	≤1	≤1	≤0.25	≤1	128	≤20
PA004	≥32	≥32	≤4	≤4	>64	≥64	≥64	≥64	≥4	≥8	32	≤1	≤1	≤2	≤1	≤1	≤0.25	≤1	64	≤20
PA004 (+IPTG)	≥32	≥32	≤4	≤4	>64	≥64	≥64	≥64	≥4	≥8	32	≤1	≤1	≤2	≤1	≤1	≤0.25	≤1	128	≤20

AMP, Ampicillin; AMC, Amoxicillin-Clavulanic acid; PIP, Piperacillin; TZP, Piperacillin-Tazobactam; CEF, Cephalothin,; CFZ, Cefazolin; CFZ, Cefuroxime axetil; FOX, Cefoxitin; CFM, Cefixime; CPD, Cefpodoxime; CTX, Cefotaxime; CAZ, Ceftazidime; IPM, Iminpenem; AMK, Amikacin; GEN, Gentamicin; TOB, Tobramycin; CIP, Ciprofloxacin; TET, Tetracycline; NIT, Nitrofurantoin; SXT, Trimethoprim-sulfamethoxazole.

Table 2. List of primers used in this study

Target	Primer name	Sequence*
A1S_0255	Ab0255_Kp_F_N	5' -CAACAGTTGGTACCAGATCAGC- 3'
	Ab0255_St_R	5' -TAGGCCTTAAAACACATCAATC- 3'
A1S_3219	Ab3219_Kp_F	5' -CTATAGGTGGGGTACCCAAA- 3'
	Ab3219_St_R	5' -CCAACCCAAGGCCTTTGAGAAT- 3'

*Genetically engineered restriction sites *KpnI* and *StuI* are underlined.

Cloning of *A1S_0255*

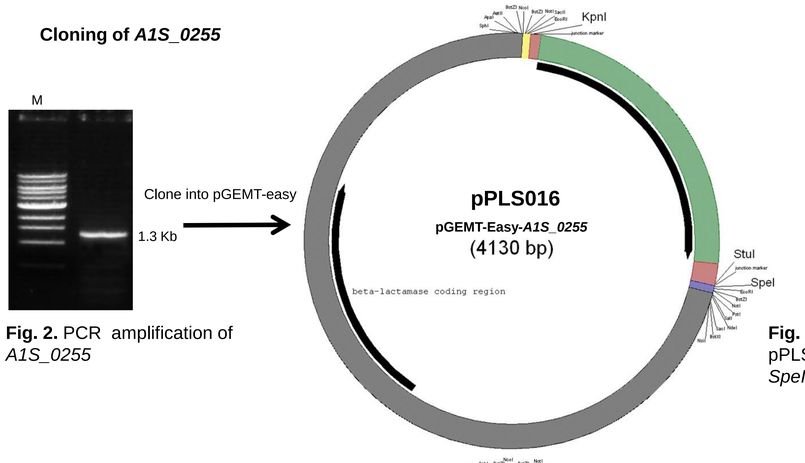


Fig. 2. PCR amplification of *A1S_0255*

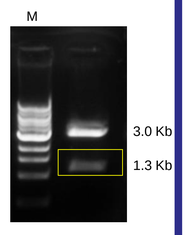


Fig. 3. Digestion of pPLS016 with *KpnI* and *SpeI*

Cloning of *A1S_3219*

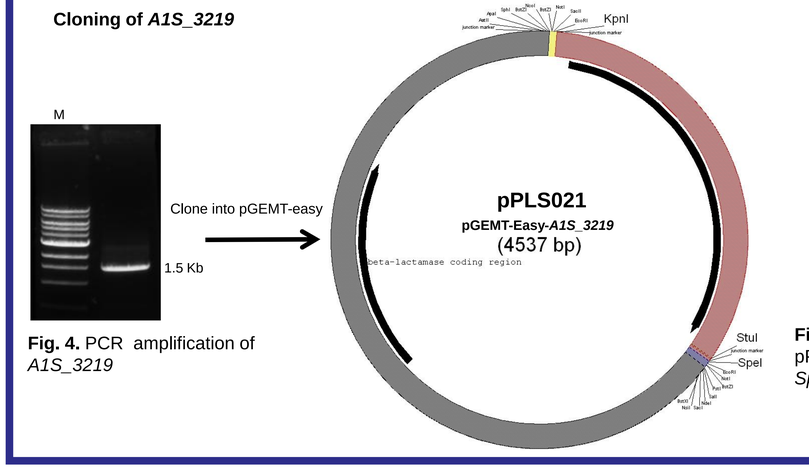


Fig. 4. PCR amplification of *A1S_3219*

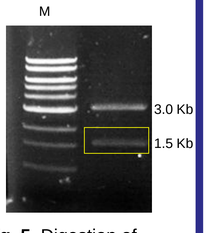
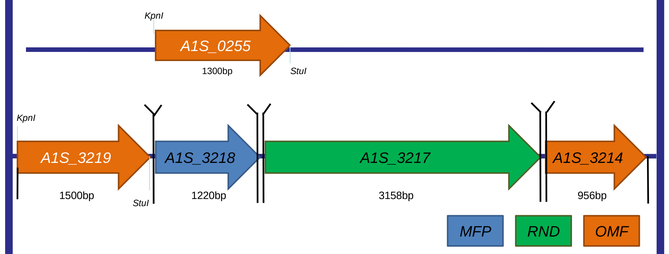
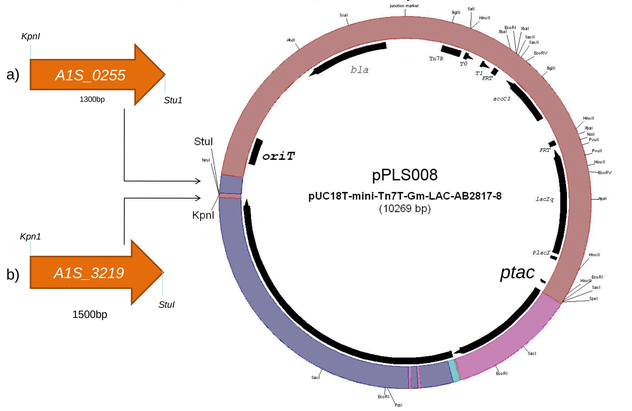


Fig. 5. Digestion of pPLS021 with *KpnI* and *SpeI*

5. Genetic organization of *A1S_0255* and *A1S_3219*



6. Insertion of *A1S_0255* and *A1S_3219* into pPLS008



RESULTS & FUTURE WORK

1. *A1S_0255 and A1S_3219* were successfully amplified and cloned into pGEMT-easy vector and confirmed by DNA sequencing.
2. Future objectives include:
   a. Clone *A1S_0255* and *A1S_3219* into pPLS008 downstream of *A1S_2817-A1S2818*.
   b. Insert the above into *P. aeruginosa* PAO750.
   c. Minimum Inhibitory Concentration (MIC) values will be determined for various antimicrobials to identify the substrates of *A1S_2817-A1S_2818-A1S_0255* and *A1S_2817-A1S_2818-A1S_3219* pumps.

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