Expression of *Pseudomonas aeruginosa* PAO1 Chitinase C in *Escherichia coli* BL21 (DE3)

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REVISED MANUSCRIPT SUPPLEMENTAL MATERIALS

Table 1S. Modifications of adapted protocols.

Transformation of plasmid DNA into competent cells (Chang et al., 2017)	500 ng of four verified *chiC*(+) 5:1 ligation transformant colony plasmids were added to four 1.5mL microcentrifuge tubes each containing 50µL of competent BL21 and incubated 30 mins on ice. The cells were heat-shocked for 45 seconds to induce plasmid uptake in a 42°C water bath. 500µL pre-warmed LB broth was added to each tube and cells were incubated for 1-hr recovery at 225rpm, 37°C. Cells were concentrated by resuspending in 300µl. 200µl aliquots were then plated on LB-kanamycin and grown overnight at 37°C.
Fast IPTG induction of subcultured *E. coli* BL21 (Biologics International Corp)	From each of the four subculture flasks, 1mL aliquots were transferred to each of 6 microcentrifuge tubes: i) uninduced BL21 lysate, ii) 1hr-induced lysate, iii) 2hrs-induced lysate, iv) uninduced BL21 supernatant, v) 1hr-induced cell supernatant, vi) 2hrs-induced supernatant. Uninduced controls were taken straight from subculture flasks and left untreated, while 1mL of 10mM IPTG was added to the 1mL induced samples. Uninduced controls were centrifuged at maximum speed (16000rpm) for 5 mins. Induced BL21 suspensions were centrifuged after 1 hour and 2 hours. 75µL of 2x Laemmli dye (Bio-Rad) with BME and 75µL of 2x Laemmli dye without BME was added to each of the pellets, and 15µL of 2x Laemmli dye with BME were added to 15µL aliquots of the supernatants. The microcentrifuge tubes were vortexed and placed in the heating block at 96°C for 5 minutes. Pellet lysate tubes were then centrifuged for 5 minutes at 16000 rpm.

Table 2S. Percent sequence alignment of Sanger sequencing reads to PAO1 *chiC*. Reads were trimmed, and regions of higher base call accuracy (quality score > 40) were aligned against the *Pseudomonas aeruginosa* PAO1 complete genome (NC_002516.2) on NCBI BLAST. Reads from forward universal primers (T7 F) and reverse internal primers (Int R) covered nucleotides 5179 to 5845 of *chiC* (NP_250990.1). Reverse universal primers (T7 R) and forward internal primers (Int F) covered nucleotides 5815 to 6610 of *chiC*.

	% identity to *chiC* nt 5179 to 5845					% identity to *chiC* nt 5815 to 6610			
	pA1	pA3	pB1	pB4		pA1	pA3	pB1	pB4
T7 F	99.4	97.74	99.85	99.55	T7 R	99.62	99.25	99.75	98.49
Int R	99.4	99.85	99.85	100	Int F	99.25	98.87	99.87	93.98

Table 3S. Summary of *E. coli* DH5α colony counts on LB+25µg/ml Kan agar from Fig 2B.

	***E. coli* DH5α cells transformed with:**				
	5:1 insert to vector ligation mixture	**3:1 insert to vector ligation mixture**	**No DNA; H_2O only (-ve ctrl)**	**Undigested, intact vector (pET-28a)**	**Double-digested pET-28a, (no insert)**
Plate A	7 kan^R colonies ↓ labeled 5:1 A1, A2, … A7	4 kan^R colonies	x no colonies	hundreds of colonies	x no colonies
Plate B	4 kan^R colonies ↓ labeled 5:1 B1, B2, B3, B4	x no colonies		hundreds of colonies	x no colonies

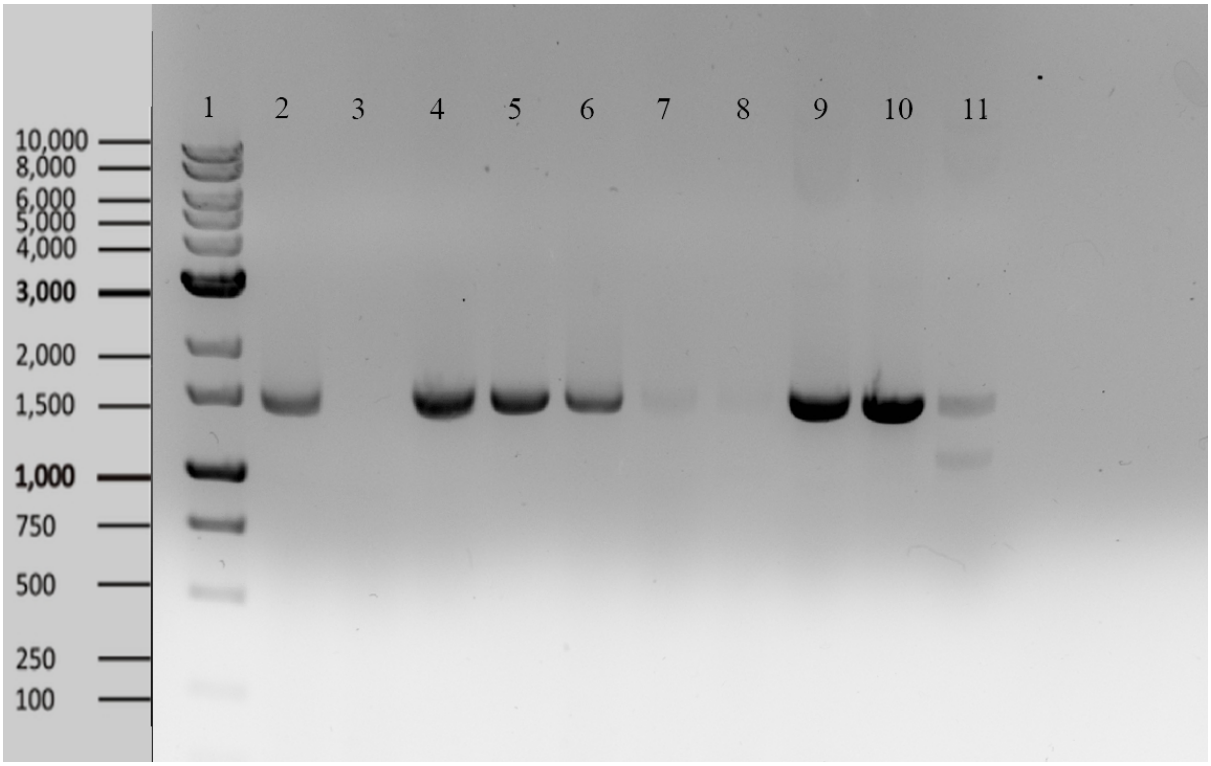

FIG 1S. Colony PCR screen of ligation product Kan^R DH5α colonies showed that 5:1 insert-to-vector ligation produced vectors containing the ~1.5kb *chiC* insert. DH5α cells transformed with candidate plasmids were added to the PCR mixture, and products of amplification with 2γ-3chiC primers showed DNA bands of the expected size. Lane 1 contains the 1Kb Plus ladder. Lanes 2-6 contain PCR products from 5:1 colonies A1, A3, A7, B1, B4. Lanes 7-8 contain PCR products from 3:1 colonies 1 and 4. Lanes 9-10 contain amplicons from pGKMS21 +ve control template. Lane 11 contains contaminated -ve control.

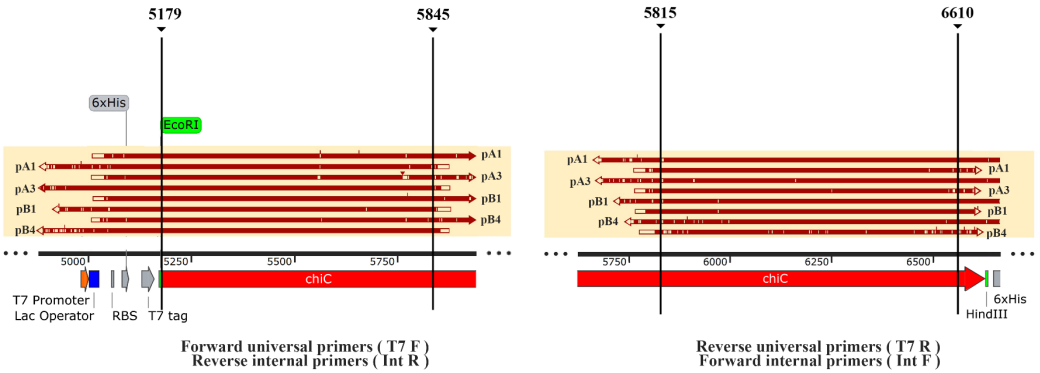

FIG 2S. Vertical lines show where Sanger sequencing reads were trimmed to exclude the terminal regions of poor quality due to sequencing error. Resulting sequences covered *chiC* (NP_250990.1) regions from nt5179 to nt5845 and nt5815 to nt6610. Query sequences were aligned against the *P. aeruginosa* PAO1 genome (NC_002516.2) on NCBI BLAST.

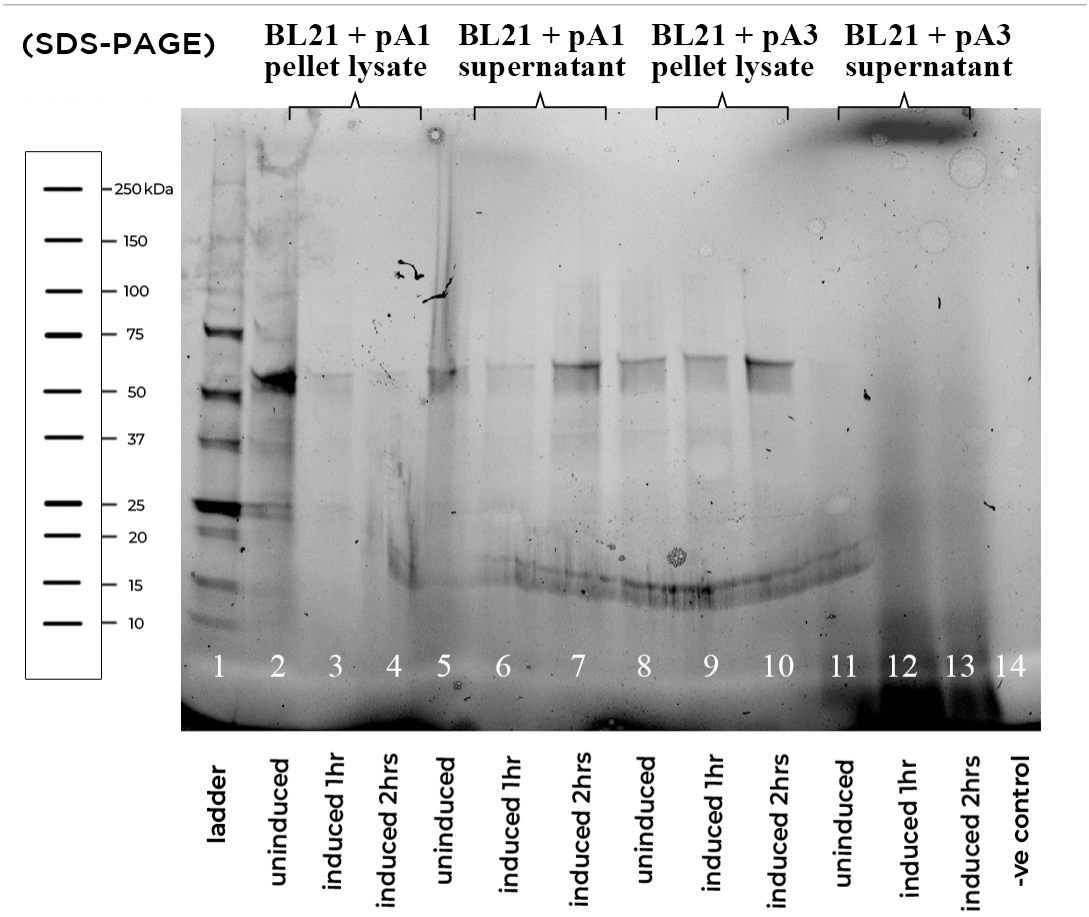

FIG 3S. Lysates and supernatants of *E. coli* BL21 transformed with verified pM3CRYY (pA1, pA3) contain varying amounts of a 55kDa protein with no clear induction pattern after IPTG treatment. Lane 1 contains the 10µl of 10-250kDa protein ladder. Lanes 2-4 and 8-10 contain proteins from pellets of BL21 subcultures that were uninduced, induced for 1 hour, and induced for 2 hours respectively. Lanes 5-7 and 11-13 contain proteins from supernatants of BL21 subcultures subject to the same conditions. Lane 14 contains the negative control.