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Investigating the chitin-binding capability of recombinant ChiC expressed in *E. coli*

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SUMMARY The chitinase C protein (ChiC), natively expressed by *Pseudomonas aeruginosa* PAO1, has been explored as a safe and environmentally-friendly crop protection agent. Previous work has been conducted to clone the *chiC* gene into pET-28a to create pM3CRYY A3, an inducible expression vector for ChiC with an N-terminal 6xHis-tag for purification. In this study, we expressed ChiC in *Escherichia coli* BL21 (DE3) transformed with the plasmid and purified the expressed protein using nickel-resin immobilized metal affinity chromatography. We then assessed chitin-binding ability using a chitin resin and showed that recombinant ChiC expressed in *E. coli* can bind to chitin *in vitro*. Our findings enable further research on the activity, stability, and applications of recombinant chitinases.

INTRODUCTION

In order to protect crops from pests, weeds, and diseases, the agriculture industry typically utilizes chemical pesticides. Although effective in ensuring the safety of the crops, many of these compounds have detrimental effects on people's health and the environment (1). It has become increasingly necessary to develop a more sustainable and ecological method of protecting crops, all while not compromising effectiveness. One such solution to combat the loss of crops from plant-feeding insect pests is to develop a natural enzyme-based pesticide that targets crucial metabolic processes and structural components in agricultural pests.

Research has shown that *Pseudomonas-derived* bioactive compounds such as mycolytic enzymes can be insecticidal against phytopathogenic fungi and plant larvae (2). Srividya et al. (2012) discovered that *Pseudomonas sp*. were effective in killing phytopathogens through the production and secretion of glycanases, proteases, and chitinases (3). Chitin is the second most abundant natural polysaccharide and the primary structural component of arthropod exoskeletons. It is a linear polysaccharide homopolymer of β-1,4-N-acetyl-D-glucosamine (4, 5). Therefore, investigating chitinases, which specifically target and break down the glycosidic linkages in chitin, could be beneficial in developing a chitinolytic pesticide. Chitinase C (ChiC), a 55-kDa secreted protein, contains a carbohydrate-binding module that facilitates the binding of chitin and a chitinolytic activity domain (GH18) which catalyzes the cleavage of β-1,4-bonds in chitin (6). Bacteria of the *Pseudomonas* genus, specifically *Pseudomonas aeruginosa* PAO1, have been shown to express ChiC (7), which is secreted into the extracellular space through a novel mechanism not involving type II or III secretion systems (8). The expression and isolation of ChiC with chitinolytic activity can be exploited to develop an effective biopesticide.

Rocha et al. (2022) constructed an inducible expression vector (pM3CRYY A3) containing the *P. aeruginosa* PAO1 *chiC* gene (4). This was accomplished by amplifying *P. aeruginosa* PAO1-derived *chiC* from the pGKM21 plasmid, which was subsequently cloned into a pET-28a(+) vector via restriction digest cloning (4). The plasmid was successfully transformed into *Escherichia coli*, a non-chitinolytic host, and expressed. However, ChiC was

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not purified and its binding capability was not investigated (4). Our study aimed to build upon the previous work done by Rocha et al. (4) using the previously constructed pM3CRYY A3 expression plasmid to express, purify, and investigate the binding ability of ChiC. We expressed ChiC in *E. coli* BL21 (DE3) cells and purified the protein using a nickel-charged affinity resin. This purification system is based upon the high affinity of nickel for 6xHis-tagged recombinant proteins (9). Purified ChiC was able to bind to the chitin resin (10), demonstrating the presence of the functional chitin-binding site, GH18. The presence of a functional chitin-binding domain provides promising results for future downstream applications involving chitinolytic activity.

METHODS AND MATERIALS

Preparation of Growth Media and Antibiotics. LB broth was prepared using distilled water (dH_2O) with 10 g/L NaCl, 10 g/L Tryptone, and 5 g/L yeast extract. 15 g/L agar was added to LB broth to prepare LB agar. The solutions were sterilized by autoclaving. The 50 mg/mL kanamycin solution was prepared with 0.6 g of kanamycin sulfate in 12 mL of dH_2O, filter sterilized (0.20 µm), and stored at 4 °C.

Isolation of the pM3CRYY A3 Plasmid from DH5α *E. coli* Cells. One colony of pM3CRYY A3 DH5α *E. coli* was inoculated into 5 mL LB supplemented with 50 µg/mL of kanamycin and grown overnight at 37 °C, 225 RPM. The plasmid was isolated using the BioBasic EZ-10 Spin Column Plasmid DNA Miniprep Kit according to the manufacturer's specifications (11) and stored at -20 °C. The concentration of the plasmid was determined using the Thermo Scientific NanoDrop™ 2000c (12).

Sanger Sequencing of the pM3CRYY A3 Plasmid. The sequencing reactions were prepared according to Genewiz's sample preparation guidelines (13) for 6-10 kb plasmids and sent for Sanger Sequencing with T7 universal and custom internal primers (Supplementary Table S1). All primers produced reads that passed quality control parameters (Supplemental Table S2). The Sanger sequencing results were analyzed using NCBI BLAST alignment (14) and SnapGene (15) (Supplemental Table S3). Sequencing reads were successfully aligned and compared against a *P. aeruginosa* PAO1 reference genome (NC_002516.2) (Supplemental Figure S1), which confirmed the presence of the intact *chiC* gene within the pM3CRYY A3 plasmid.

Preparation of competent BL21 *E. coli* cells. The competent cells were prepared following an open-access protocol (16). One colony of *E. coli* BL21 (DE3) was inoculated into 5 mL of LB and grown overnight at 37 °C, 225 RPM, before being subcultured 1/100 into 100 mL of LB. The culture was pelleted at an OD_{600} of 0.5. After resuspension in 25% original volume (25 mL) of cold 100 mM $MgCl_2$ and incubation on ice for 5 mins, the culture was spun again and the supernatant was decanted. This was repeated using 5% original volume (5 mL) of cold 100 mM $CaCl_2$, with incubation on ice for 20 min instead. The final pellet was resuspended in 1% original volume (1 mL) of a cold 85% v/v 100 mM $CaCl_2$ and 15% v/v glycerol solution before being aliquoted and stored at -70 °C.

Transformation of *E. coli* BL21 (DE3) with the pM3CRYY A3 plasmid. 50 ng of plasmid was added to 40 µL of competent BL21 cells. Both cells and plasmid DNA were incubated on ice for 1 hr. The cells were heat shocked at 42 °C for 25 seconds and warm LB was added before incubation for 1 hr at 37 °C, 225 RPM. 100 µL of the transformants and negative control were plated at various dilutions onto LB agar plates supplemented with 50 µg/mL of kanamycin and incubated overnight at 37 °C.

ChiC Expression in *E. coli* BL21 (DE3). One colony of pM3CRYY A3 *E. coli* BL21 transformant was inoculated into LB supplemented with 50 µg/mL of kanamycin and the culture was incubated overnight at 37 °C, 225 RPM. The overnight culture was subcultured 1/100 into 250 mL of LB supplemented with 50 µg/mL of kanamycin and incubated at 37 °C, 225 RPM. At an OD_{600} of 0.70, the culture was induced with 1 mM of IPTG and incubated

at 25 °C, 225 RPM for 20 hrs before being spun for 10 min at 5,000 RPM. An aliquot of the supernatant was taken and the pellet was stored at -70 °C until cell lysis and protein extraction. The above procedures were repeated using a pET-28a vector-only transformant, except the overnight culture was subcultured 1/200 into 200 mL of media and induced at an OD_{600} of 0.67.

Cell Lysis, Protein Extraction, and Expression Testing. The induced pellets of the pM3CRYY A3 and pET-28a control cultures (2.5 g and 1.6 g, respectively) were each resuspended in 12.5 mL of lysis buffer composed of 50 mM Na_2HPO_4, 300 mM NaCl, 1% Triton X-100 and 10% glycerol, with 0.01% β-Me, 1 mg/mL lysozyme, and 1 tablet/12.5 mL of Roche cOmplete™ Mini Protease Inhibitor Cocktail Tablet added fresh and the pH adjusted to 7.5. After stirring on ice for 30 min, the cell lysates were passed through a 26-gauge needle five times. The whole cell lysates were spun for 10 min at 5,000 RPM and the supernatants (soluble fractions) were stored at -20 °C until protein purification. Aliquots of the samples were taken after each step. The supernatants, whole cell lysates, soluble fractions, and final pellets for the pM3CRYY A3 and pET-28a control cultures were analyzed by SDS-PAGE.

ChiC Purification using Nickel-Charged Affinity Resin. Purification of ChiC was performed using the NEBExpress® Ni Resin. The soluble fraction of the pM3CRYY A3 culture was passed through a 0.45 μM syringe filter before 2 mL of the filtrate was loaded onto 0.5 mL of resin previously equilibrated with 10 mL of binding buffer (50 mM Na_2HPO_4, 300 mM NaCl; pH adjusted to 7.5). The soluble fraction input was allowed to interact with the resin for 10 min before the flowthrough was collected. The resin was washed with 5 mL of 10 mM imidazole wash buffer, then 5 mL of 25 mM imidazole wash buffer. Elution was performed using 5 mL of 500 mM imidazole elution buffer and collected in 0.5 mL fractions. The wash and elution buffers were prepared by adding the respective concentrations of imidazole to the binding buffer. The filtered input, flowthrough, 10 mM wash, 25 mM wash, and ten elution fractions were analyzed by SDS-PAGE.

Sample Pooling and Dialysis. Elution fractions 2 to 8 were pooled (4 mL) and an aliquot [9.5] was taken for future assays. The samples were stored at -70 °C in 10% glycerol. After thawing, the sample was dialyzed in the Spectra/Por 4 12-14 kD MWCO membrane (17) for 24 hrs in 2 L of binding buffer. The dialyzed sample (3 mL) was split into two aliquots [9.7.1, 9.7.2] and stored at -70 °C in 15% glycerol.

Chitin Resin. NEB chitin resin (10) was equilibrated in 5x volume of binding buffer. 100 uL of resin were each incubated with 250 μL of protein samples (pre-dialysis ChiC [9.5], post-dialysis ChiC [9.7.1], and 1 mg/mL bovine serum albumin control) at room temperature with shaking. The resins were centrifuged at 7,500 RPM and the supernatants (flow throughs) were collected. The resins were washed with 500 μL of binding buffer by incubation for 5 minutes at room temperature, with shaking, followed by centrifugation and collection of supernatants (washes). Two additional washes were performed. For elution, 200 μL of Laemmli buffer (18) was added to the resins, which were then boiled for 5 min at 95 °C before centrifugation and collection of supernatants (eluents). The remaining resins were resuspended in 150 μL of Laemmli buffer. The samples were stored at -20 °C before SDS-PAGE analysis.

BCA Assay. Protein quantification was performed using the Pierce™ BCA Protein Assay Kit (19). 25 μL of samples (triplicates) and BSA standards (duplicates) were added to each well in serial dilutions, followed by 200 μL of working solution (20 mL BCA solution A, 0.4 mL copper solution). The plate was incubated at 37 °C for 30 min, with shaking. The absorbances were measured at 562 nm using the BioTek Epoch Microplate Spectrophotometer (20). The protein concentrations were calculated using Microsoft Excel.

RESULTS

pM3CRYY *E. coli* BL21 (DE3) expressed a 55-kDa protein after induction with IPTG. SDS-PAGE analysis was performed to determine whether *E. coli* BL21 (DE3) transformed with pM3CRYY A3 expressed ChiC following IPTG induction. During the pelleting and lysis processes, the supernatant, whole-cell lysate, soluble fraction, and pellet samples from both the pM3CRYY experimental culture and the pET-28a vector-only control culture were collected. The overall protein concentrations were much higher in the experimental culture transformed with the pM3CRYY plasmid versus the vector-only control (Figure 1). Comparing across sample types for both cultures, the soluble fractions had the highest concentration of proteins, followed by the whole cell lysates and the pellets, while the supernatants contained no detectable amounts of proteins (Figure 1). Both the control and experimental cultures' whole-cell lysate and soluble fraction samples contained proteins of various molecular weights (Figure 1). However, there was a notably high concentration of an approximately 55-kDa protein in the experimental whole-cell lysate, with an even higher concentration in the experimental soluble fraction. This protein was still seen in relatively high concentration, compared to proteins of other molecular weights, in the experimental pellet (Figure 1). Meanwhile, the whole cell lysate, soluble fraction, and pellet of the pET-28a vector-only control culture did not contain especially high concentrations of this 55-kDa protein (Figure 1). Therefore, it was possible to presume that this 55-kDa protein was ChiC. Altogether, the results suggested that IPTG-induced ChiC production occurs in pM3CRYY cells but not in pET-28a vector-only control cells.

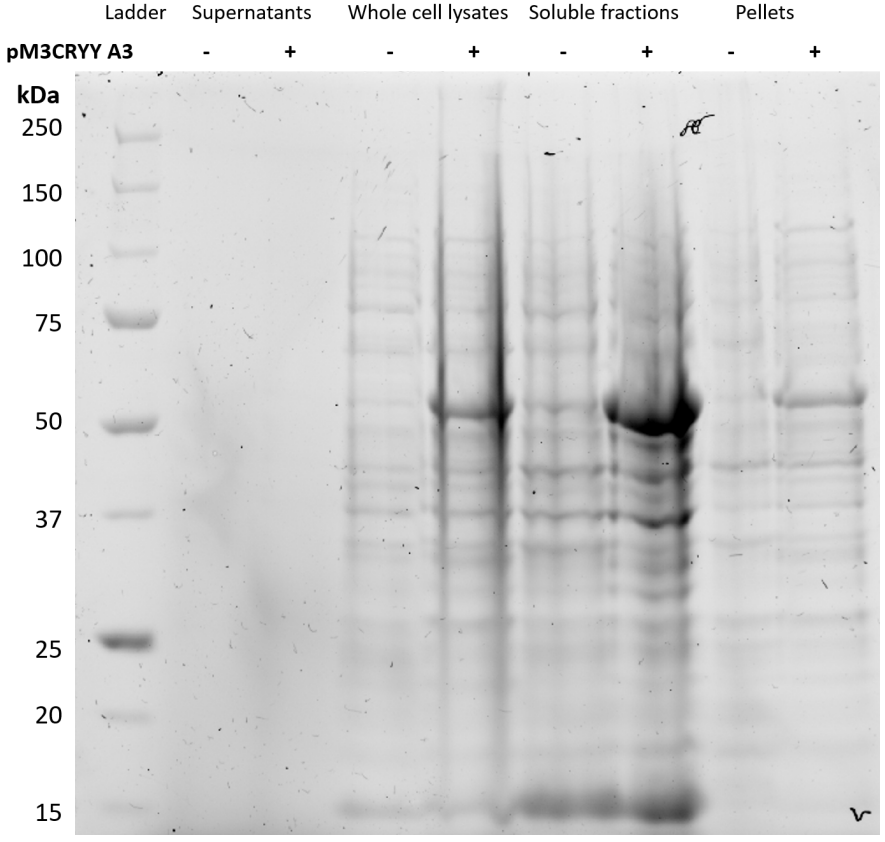

FIG. 1 SDS-PAGE results indicated the presence of a 55-kDa protein expressed in induced *E. coli* BL21 (DE3) cells transformed with pM3CRYY A3. The gel was loaded with the supernatants, whole cell lysates, soluble fractions, and final pelletsF of the induced pM3CRYY A3 (+) or induced pET-28a vector-only (-) *E. coli* cultures. Samples were run on a 10% SDS-PAGE gel for 45 minutes at 200 V. The gel was visualized using the ChemiDoc™ Imaging System.

ChiC was successfully purified using immobilized metal affinity chromatography. In order to purify the recombinant ChiC, nickel-based immobilized metal affinity chromatography (IMAC) purification was performed to isolate His-tagged proteins. As the pM3CRYY A3 plasmid included a 6xHis-Tag on the *chiC* insert's N-terminus, the expressed ChiC was able to bind to the nickel resin with high affinity. SDS-PAGE was performed to analyze the results of the purification. The input (filtered soluble fraction) contained proteins of varying molecular weights, including the 55-kDa protein presumed to be ChiC (Figure 2). The flowthrough had a similar protein profile, except for a reduced concentration of the 55-

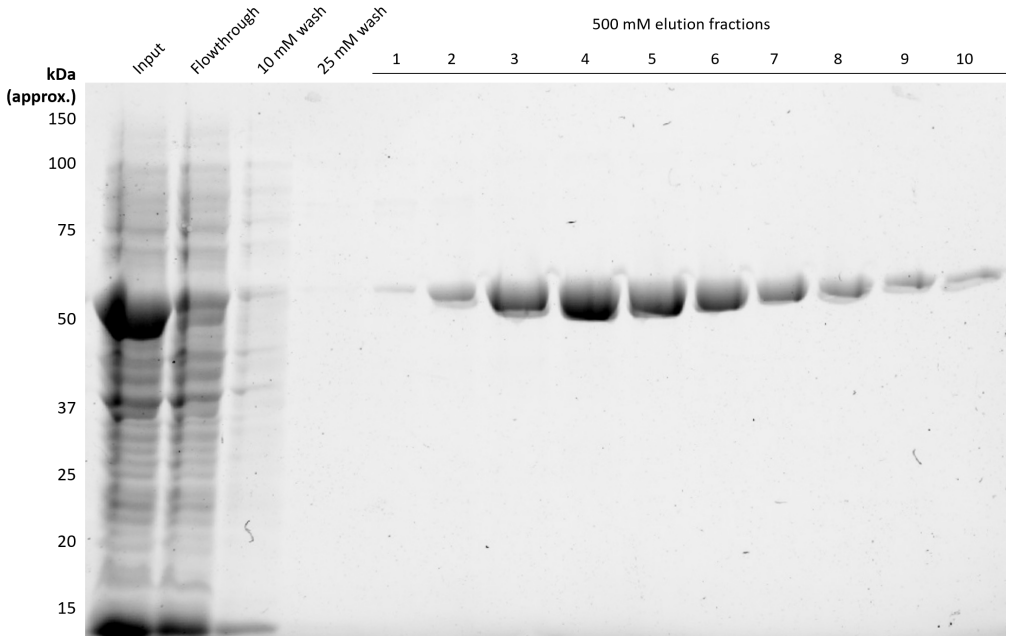

FIG. 2 Recombinant ChiC expressed in *E. coli* BL21 (DE3) was successfully purified by nickel-based IMAC. An SDS-PAGE gel was loaded with 15 µL of filtered soluble fraction (input), flowthrough, 10 mM wash flowthrough, 25 mM wash flowthrough, and 500 mM elution fractions resulting from the purification. Samples were run on a 10% SDS-PAGE gel for 45 minutes at 200 V. The gel was visualized using the ChemiDoc™ Imaging System. The protein ladder was omitted as it was faulty and not visible. The labeled molecular weights are estimates.

kDa protein, possibly suggesting the binding of the His-tagged ChiC to the nickel resin (Figure 2). The first wash with 10 mM imidazole buffer removed much of the contaminating proteins from the resin, as indicated by the presence of proteins other than the presumed ChiC in the wash (Figure 2). There were minimal amounts of protein in the flowthrough from the 25 mM imidazole wash, suggesting that the majority of the contaminants had been removed by the previous 10 mM imidazole wash (Figure 2). The flowthrough fractions from the 500 mM imidazole elution contained high concentrations of the 55-kDa protein, without detectable amounts of contaminating proteins. This possibly suggests the isolation of reasonably pure and concentrated ChiC. Elution fractions 2-8, which contained the most protein, were pooled and dialyzed to remove the imidazole. The pooled and dialyzed samples [9.7.1, 9.7.2] were quantified by a BCA assay to contain approximately 1,150 µg/mL of protein, most of which was presumed to be ChiC (Figure 2). This corresponds to over 4.6 mg of protein expressed and purified from just 250 mL of induced E. coli culture, translating to a substantial yield of over 18.4 mg of protein per liter of culture.

Recombinant ChiC contained a functional chitin-binding domain. Chitin resin was used to demonstrate the chitin-binding ability of the purified and dialyzed ChiC. BSA, which lacks chitin-binding domains, was used as a control. SDS-PAGE analysis revealed that the BSA input and flowthrough samples were nearly identical with strong bands at 66.5 kDa, the expected weight of the protein. This indicates that minimal amounts of BSA were bound to the chitin resin after incubation (Figure 3). The first wash removed the unbound BSA from the resin, hence the faint band at 66.5 kDa (Figure 3). The elution and resin samples also displayed weak bands, further suggesting that BSA was unable to bind to the chitin resin (Figure 3). Conversely, the ChiC flowthrough showed a thinner band at 55 kDa than the input, suggesting that some ChiC had bound to the chitin resin (Figure 3). The three washes with binding buffer also washed some of the ChiC from the resin, shown by the faint bands at 55 kDa (Figure 3). The eluted sample generated a band of similar thickness and intensity as the input, which further suggests that ChiC has a functional chitin-binding domain (Figure 3). After elution, the resin still displayed an intense band at 55 kDa, indicating that much of the ChiC remained bound to the chitin resin (Figure 3).

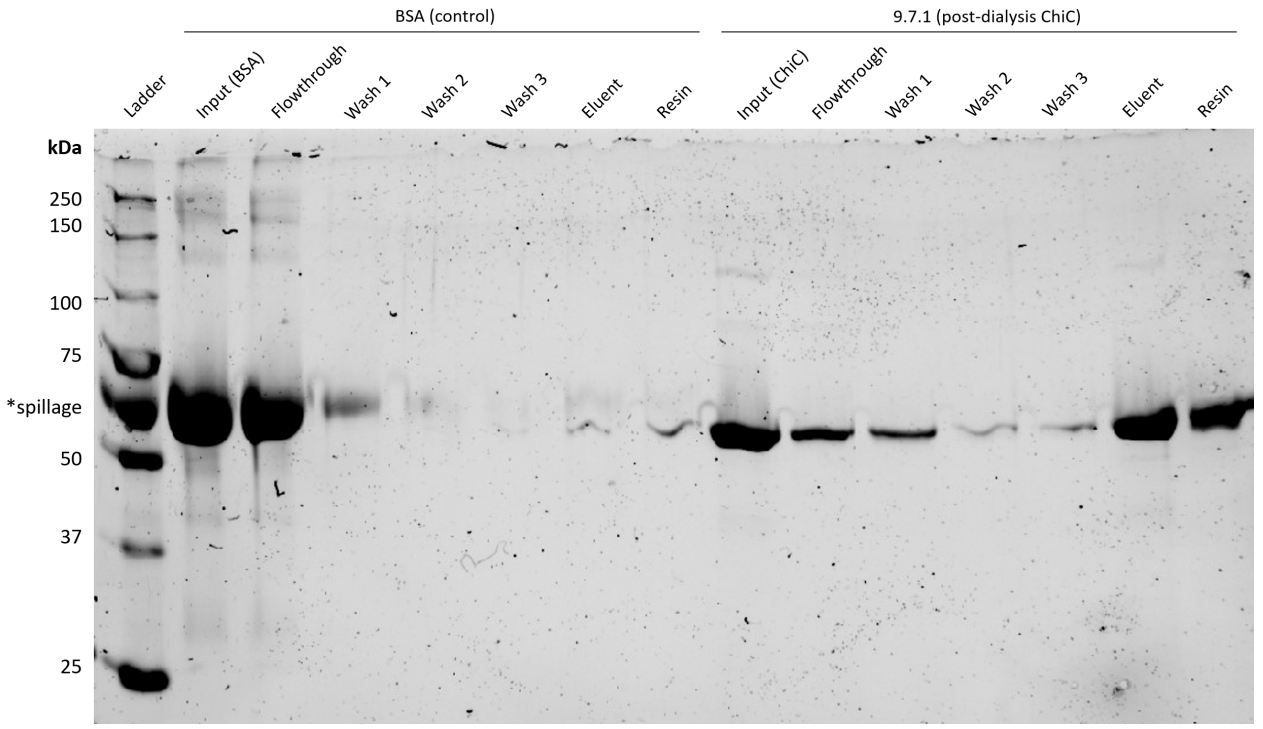

FIG. 3 The purified ChiC contained a functional chitin-binding domain. The chitin-binding abilities of ChiC and BSA were compared using chitin beads. The input, flowthrough, washes, elution, and resin samples were loaded and run on a 10% SDS-PAGE gel for 45 minutes at 200 V. The gel was visualized using the ChemiDoc™ Imaging System. The band between 50-75 kDa on the protein ladder was spillage from the BSA input.

DISCUSSION

The primary objectives of this study were to transform *E. coli* BL21 (DE3) with the previously constructed pM3CRYY A3 plasmid, induce the expression of ChiC, purify the recombinant protein, and test the functionality of its chitin-binding domain. To accomplish this, the identity of the pM3CRYY A3 plasmid was confirmed by Sanger sequencing (Methods: Sanger Sequencing). Then, *E. coli* BL21 (DE3) cells were transformed with the plasmid, and the expression of ChiC was successfully induced using IPTG. Nickel-based IMAC was used to purify the recombinant protein and the chitin-binding ability of the purified ChiC was examined using a chitin resin.

After induction, the ChiC-expressing *E. coli* cells were chemically and physically lysed. Various samples were collected throughout the process to determine the presence and solubility of ChiC. The presence of ChiC in the last elution fraction suggests that some ChiC remained on the resin, and further elution with 500 mM imidazole could potentially increase the overall protein yield (Figure 2). Additionally, the flowthrough sample still contained some ChiC, which could be recovered by either re-loading it onto the resin prior to washing or increasing the binding time (Figure 2). Meanwhile, the low concentration of proteins, including ChiC, in the final pellets suggests that the lysis procedure was effective in lysing the cells and extracting all the cellular proteins (Figure 1). In *P. aeruginosa*, the naturally expressed ChiC was found in the supernatant after several days of growth (8). However, secretion of recombinant ChiC in *E. coli* is unlikely, since ChiC appears to be secreted via an uncharacterized secretion pathway, which may be encoded by unique genes in *P. aeruginosa* (8). Indeed, SDS-PAGE analysis indicated that the 55-kDa protein was primarily detected within the soluble fractions of the lysed *E. coli* rather than the supernatant (Figure 1). However, an overnight induction may not provide sufficient time for ChiC secretion, which is limited even in *P. aeruginosa* for the initial two days (8). Additionally, the presence of ChiC in the supernatant cannot be ruled out due to the large volume of the collected sample, which would dilute all secreted proteins. No bands were seen for the supernatant samples, indicating that even secreted native proteins were too dilute to be detected. Further processing

of the supernatant samples by protein ultrafiltration with a membrane of appropriate molecular-weight cutoff is required to determine whether *E. coli* is able to secret recombinant ChiC.

Recombinant ChiC was determined to have a functional chitin-binding domain. Prior studies have reported that the native ChiC produced by *P. aeruginosa* contains a chitin-binding domain in addition to its chitinolytic domain, which operates synergistically to degrade chitin (8). As such, testing the recombinant protein's chitin-binding capabilities is crucial in evaluating its complete functionality. The purified ChiC was shown to successfully bind to the chitin resin, withstanding several washes and being eluted only after the addition of Laemmli buffer coupled with boiling. SDS-PAGE analysis also showed that large amounts of the protein remained on the resin after elution, suggesting a strong interaction with the chitin resin (Figure 3). Interestingly, the chitin-binding ability of the purified protein was similar both before and after dialysis, indicating that 500 mM imidazole did not inhibit the binding of ChiC to the chitin resin (Supplemental Figure S2). This suggests that either the structure of ChiC was unaffected by high concentrations of imidazole, or that the chitin-binding domain was still functional and extracellular in the imidazole-denatured protein. However, it should be noted that a functional chitin-binding domain alone does not indicate chitinolytic activity. Thus, chitin-cleavage enzymatic activity assays must be conducted in order to determine whether the recombinant ChiC is able to degrade chitin.

Limitations While this study provides evidence that recombinant ChiC has a functional chitin-binding domain, a few key limitations should be noted. First, the *P. aeruginosa* PAO1 genome has a higher average GC content in the third codon position compared to *E. coli*, which could alter expression levels due to differential preference in codon usage (22). Therefore, extrapolating the results observed from the ChiC expression in *E. coli* BL21 to a *P. aeruginosa* model system may not be accurate. In addition, we tested only one induction condition for ChiC expression using 1 mM of IPTG at 25°C for 20 hours. Although our results showed ample expression of ChiC, other IPTG concentrations, temperatures, and induction durations should be tested to maximize protein yield.

Conclusions In this study, we successfully expressed ChiC in *E. coli* before proceeding with its purification, quantification, and assessment of chitin-binding ability. We show that *E. coli* BL21 (DE3) transformed with pM3CRYY A3 expresses recombinant ChiC following IPTG induction. Using nickel-based IMAC purification, we were able to obtain a pure ChiC sample at a concentration of 1150 µg/mL. Finally, we establish that this purified recombinant ChiC is able to bind to chitin *in vitro.*

Future Directions In this experiment, the enzymatic activity of the purified ChiC product has not been determined. In order to confirm the chitinolytic activity of the protein, an experiment using colloidal chitin plate assays could be conducted. This would involve plating induced pM3CRYY *E. coli* or purified ChiC and measuring the plate clearing to assay for chitinolytic activity. Moreover, the *in vivo* enzymatic activity of ChiC could be confirmed using an insecticidal or antifungal assay. The *Spodoptera litura* larvae assay is a potential bioassay (23). In addition, the expression and purification processes can be further refined to increase the yield and purity of the product, which may be required for future applications such as activity assays or X-ray crystallography. Finally, further studies are needed to determine the most effective way to store purified ChiC to preserve its structure and function. For instance, experiments could be conducted to determine the optimal percentage of glycerol and other reagents such as DTT to add to the storage solution and whether flash freezing with liquid nitrogen is necessary. After the enzymatic activity of recombinant ChiC is established, potential applications of the protein should be investigated.

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CONTRIBUTIONS

Laboratory work performed by L.L., P.A., J.T., and S.R. Abstract by L.L. Introduction primarily by J.T., with contributions from P.A. and S.R. Materials and Methods primarily by S.R. and L.L., with contributions from P.A. Results primarily by P.A. and L.L., with contributions from J.T. Discussion primarily by L.L. and P.A., with contributions from J.T. Limitations, Conclusions, and Future Directions primarily by J.T., with contributions from S.R. and L.L. Figures by L.L. Figure legends by P.A. References compiled by G.S. Draft pre-review edits by all authors. Final pre-publication edits by L.L., J.T., S.R., and P.A.

REFERENCES

1. Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L. 2016. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Front Public Health 4:148. doi: 10.3389/fpubh.2016.00148.
2. Mnif I, Ghribi D. 2015. Potential of bacterial derived biopesticides in pest management. Crop Protection 77:52-64. https://doi.org/10.1016/j.cropro.2015.07.017.
3. Srividya S, Ramyasmruthi S, Pallavi O, Pallavi S, Tilak K. 2012. Mycolytic enzymes of fluorescent Pseudomonas sp. R as effective biocontrol against Colletotrichum gloeosporoides OGC1. Asiatic Journal of Biotechnology Resources 03(10):1425-1433.
4. Rocha MC, Yap ME, Yoon M. 2022. Expression of Pseudomonas aeruginosa PAO1 Chitinase C in Escherichia coli BL21 (DE3). UJEMI 27:1-10.
5. Shahidi F, Abuzaytoun R. 2005. Chitin, chitosan, and co-products: Chemistry, production, applications, and health effects. In: Advances in Food and Nutrition Research. Elsevier Science & Technology 49:93-135.
6. Liu J, Xu Q, Wu Y, Sun D, Zhu J, Liu C, Liu W. 2023. Carbohydrate-binding modules of ChiB and ChiC promote the chitinolytic system of Serratia marcescens BWL1001. Enzyme Microb Technol. 162:110118.
7. Chan N, Pannu S, Xiong L, Yeung D. 2022. Construction of inducible expression vector pDNLS-22n containing P. aeruginosa PAO1 ChiC. UJEMI 27:1-11.
8. Folders J, Algra J, Roelofs MS, van Loon LC, Tommassen J, Bitter W. 2001. Characterization of Pseudomonas aeruginosa chitinase, a gradually secreted protein. J Bacteriol 183:7044–7052.
9. Life Technologies Corporation. 2015. Ni-Nta Purification System - Thermo Fisher Scientific. https://tools.thermofisher.com/content/sfs/manuals/ninta_system_man.pdf
10. New England BioLabs Ltd. 2020. Chitin Resin. https://www.neb.ca/S6651
11. Bio Basic Inc. 2017. EZ-10 Spin Column Plasmid DNA Miniprep Kit. https://www.biobasic.com/ez-10-spin-column-plasmid-dna-miniprep-kit-4155
12. ThermoFisher Scientific Inc. 2022. NanoDrop™ 2000/2000c Spectrophotometers. https://www.thermofisher.com/order/catalog/product/ND2000CLAPTOP
13. Genewiz. 2022. DNA Sequencing Sample Submission Guidlines. https://www.genewiz.com/Public/Resources/Sample-Submission-Guidelines/Sanger-Sequencing-Sample-Submission-Guidelines/Sample-Preparation/
14. National Library of Medicine. 2022. Nucleotide BLAST. https://blast.ncbi.nlm.nih.gov/Blast.cgi?BLAST_SPEC=blast2seq&LINK_LOC=align2seq&PAGE_TYPE=BlastSearch
15. SnapGene. 2022. Plasmid Files. https://www.snapgene.com/
16. Scientific Protocols. 2015. Preparation of Competent Cells. https://protocols.scienceexchange.com/protocols/preparation-of-competent-cells
17. Fisher Scientific. 2022. Spectrum™ Spectra/Por™ 4 RC Dialysis Membrane Tubing 12,000 to 14,000 Dalton MWCO. https://www.fishersci.ca/shop/products/spectrum-spectra-por-4-rc-dialysis-membrane-tubing-12-000-14-000-dalton-mwco-4/p-86966
18. Bio-Rad Laboratories Inc. 2023. 2x Laemmli Sample Buffer. https://www.bio-rad.com/fr-ca/sku/1610737edu-2x-laemmli-sample-buffer?ID=1610737EDU
19. ThermoFisher Scientific Inc. 2022. Pierce™ BCA Protein Assay Kit. https://www.thermofisher.com/order/catalog/product/23225
20. Agilent Technologies. 2022. Epoch Microplate Spectrophotometer. https://www.biotek.com/products/detection-microplate-readers/epoch-microplate-spectrophotometer
21. NCBI. 2022. Pseudomonas aeruginosa (ID 187) - Genome - NCBI. https://www.ncbi.nlm.nih.gov/genome?term=NC_002516.2.

22. Grocock RJ, Sharp PM. 2002. Synonymous codon usage in Pseudomonas aeruginosa PA01. Gene 289:131–139.
23. Zhong W, Ding S, Guo H. 2015. The Chitinase C Gene PsChiC from Pseudomonas Sp. and its Synergistic Effects on Larvicidal Activity. Genetics and Molecular Biology 38(3): 366-372. doi: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4612601/