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Expression and characterization of Pen b 26 allergen of *Penicillium brevicompactum* in *Escherichia coli*

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ABSTRACT

Pen b 26 is one of the allergens produced by *Penicillium brevicompactum* which is one of the most prevalent indoor airborne fungi and a major source of respiratory problems, including asthma. Pen b 26 was cloned and expressed as an N-terminal as well as a C-terminal His_6 tagged fusion protein in *Escherichia coli*. This allergen was purified by immobilized Ni^{2+}-affinity chromatography. The purified Pen b 26 was characterized by immunological, biochemical and biophysical methods. C-His_6 tagged Pen b 26 produced several fold greater yield than N-His_6 tagged Pen b 26. The affinity of C-His_6 tagged Pen b 26 for the specific antibody was also 2.75 times higher than N-His_6 tagged Pen b 26.

The prevalence of airborne allergic diseases has been on the rise, especially in the developed countries where the vast majority of the people live in artificially created and controlled indoor air environments [1]. This tightly regulated air is often prone to the overgrowth of ever-present mold species living indoor environments especially if the excess humidity is not controlled appropriately. The mold-overgrowth is often associated with a number of allergic symptoms involving respiratory system including asthma. *Penicillium* species have been identified as a major source of indoor mold allergies and respiratory problems including asthma and Farmer's Lung syndrome [2–6]. *Penicillium brevicompactum* is one of the most frequently isolated indoor *Penicillium* species. In addition, *P. brevicompactum* has also been reported to be one of the major food spoilage organisms and a source of mycotoxins [7–10].

Molecular characterization of allergens is of significant value to understand the pathogenesis of allergic diseases and the development of specific diagnostic and efficacious therapies. Classical methods of allergen protein purification often produce low yield of poor quality allergens. Allergens cloned using recombinant DNA technologies can be produced in large quantities with exceptional purity. Purified allergens are useful for developing diagnostic tests to screen susceptible populations allergic to mold as well as for the hyposensitization of these patients to induce the production of protective IgG antibodies. Affinity tags, which are incorporated into one end of a protein, have been frequently used for protein detection and purification. The hexa-histidine (His_6)[1] tag is one of the most popular tags. His_6 tag provides a better advantage over other tags as it binds strongly to the immobilized metal ion affinity chromatography (IMAC) resin and its small size often eliminates the need for proteolytic removal. Although His_6 tag can be incorporated into either end of the protein, N-terminal His_6 tag has been known to produce better solubility and higher yield than the C-terminal His_6 tag [11].

We have recently analyzed the extracts of 14 *Penicillium* species, including *P. brevicompactum* [12]. The extracts of *P. brevicompactum* showed at least six allergenic bands resolved by immunoblot analysis. To date, very few allergens have been isolated and cloned from *Penicillium* species. Allergen Pen b 26 has been recently isolated from a cDNA library of *P. brevicompactum* in our laboratory [13]. In this study, Pen b 26 was over-expressed using His_6 tag expression system. In order to determine the best possible expression, we employed both the N- and the C-terminal His_6 tag containing expression vectors. This paper describes the expression of Pen b 26 in *Escherichia coli* and its characterization.

Materials and methods

Penicillium brevicompactum strain DAOM 231273 (Pen-01-0001) was obtained from Canadian Collection of Fungal Cultures, Agricul-

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[1] *Abbreviations used:* His_6, hexa-histidine; IMAC, immobilized metal ion affinity chromatography; PCR, polymerase chain reaction.

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ture, and Agri-Food Canada and used to isolate total RNA and to construct a cDNA library as described earlier [13].

Construction of the expression vectors

The cDNA encoding Pen b 26 was amplified by polymerase chain reaction (PCR) using two internal forward and reverse primer pairs and cloned into two different His_6-tag containing expression vectors (Invitrogen Life Technologies, Carlsbad, California, USA): pTrcHisTOPO vector, which had a His_6 tag at the N-terminus (Fig. 1a) and pTrcHis2TOPO, which had a His_6 tag at the C-terminus (Fig. 1b). The forward primer (5′-ATGTCTACCGCTGAGCTCGCTG-3′, the first 22 nts of the protein coding sequence of the native Pen b 26) and the reverse primer (5′-GCTTAGTCGAAGAGACCGAAGC-3′, the reverse complementary sequence of the last 18 nts in the 3′end of the coding sequence, stop codon and the flanking two nucleotides of the native Pen b 26) were used for the construction of pTrcHisTOPO-Pen b 26 (N-His_6 tagged) vector. The same forward primer and a His_6 tag containing reverse primer (5′-TTAATGAT GATGATGATGATGGTCGAAGAGACCGAAGCCC-3′, the reverse complementary sequence of the last 19 nts in the 3′end of the coding sequence followed by the coding sequence of 6 His residues and the stop codon) were used for the construction of pTrcHis2TOPO-Pen b 26 (C-His_6 tagged) vector. PCR was performed by initial denaturation at 94 °C for 3 min, followed by 30 cycles of 30 s denaturation at 94 °C, 15 s annealing at 55 °C, and 30 s extension at 72 °C. A 7 min extension at the end of the 30 cycles was also incorporated. Both constructs were transformed into *E.* coli TOP10 strain (Invitrogen).

The selected PCR clones were verified by DNA sequencing as described earlier [13]. In pTrcHisTOPO-Pen b 26 construct, total of 35 residues were added to the N-terminus of the native protein which contained the HisG epitope for detection and purification of the recombinant and the original stop codon was retained. The N-His_6 tag fused peptide added about 3.8 kDa to the size of the native Pen b 26, thereby increasing its estimated size from about 11 to 14.8 kDa. In pTrcHis2TOPO-Pen b 26 construct, three external residues from the vector in the N-terminus and the six histidine residues in the C-terminus added about 1 kDa to the size of the native Pen b 26, to give a protein of 12 kDa.

Expression of Pen b 26 fusion proteins in E. coli

The cultures of *E. coli* with the appropriate expression constructs were grown in LB (Luria–Bertani) medium with 50 μg/mL ampicillin at 37 °C, 225 rpm. The expression of Pen b 26 was induced by the addition of IPTG into the culture medium at mid-log phase (A_{600nm} = 0.6) to 1 mM final concentration. For small-scale, time-course expression, the cultures of 200 mL in 1 L flasks were grown and samples of 10 mL were taken with various time intervals. The samples were centrifuged for 5 min at 10,000*g*, 4 °C. The pellets were stored at −84 °C until needed. Large scale expression was performed in 4 × 500 mL LB medium with ampicillin (50 μg/mL) in 2 L flasks for the large-scale purification

a **Pen b26 with N-terminal His_6-tag**

```
ATG GGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG
 M   G   G   S   H   H   H   H   H   H   G   M   A   S   M
ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT
 T   G   G   Q   Q   M   G   R   D   L   Y   D   D   D   D
AAG GAT CCA ACC CTT ATG TCT ACC GCT GAG CTC GCT GTC TCT TAC
 K   D   P   T   L   M   S   T   A   E   L   A   V   S   Y
GCC GCC CTC ATC CTG GCC GAT GAC GGT ATT GAG GTC TCC GCC GAC
 A   A   L   I   L   A   D   D   G   I   E   V   S   A   D
AAG ATC CAG ACC ATC CTC GGT GCC GCC AAG GTC CAG GAG GTT GAG
 K   I   Q   T   I   L   G   A   A   K   V   Q   E   V   E
CCC ATC TGG GCC ACC ATC TTC GCC AAG GCC CTC GAG GGT AAG GAC
 P   I   W   A   T   I   F   A   K   A   L   E   G   K   D
ATC AAG GAG ATC CTG ACC AAC GTC GGC TCC GCT GGT CCC GCC ACC
 I   K   E   I   L   T   N   V   G   S   A   G   P   A   T
GCC GGT GCC CCC GCC GCT GCT GGT GCC CGC GCT CCC GCT GAG GAG
 A   G   A   P   A   A   A   G   A   R   A   P   A   E   E
AAG AAG GAG GAG AAG GAA GAG GAG AAG GAG GAG TCC GAT GAG GAC
 K   K   E   E   K   E   E   E   K   E   E   S   D   E   D
ATG GGC TTC GGT CTC TTC GAC TAA GC
 M   G   F   G   L   F   D   *
```

b **Pen b26 with C-terminal His_6-tag**

```
ATG GCC CTT ATG TCT ACC GCT GAG CTC GCT GTC TCT TAC GCC GCC
 M   A   L   M   S   T   A   E   L   A   V   S   Y   A   A
CTC ATC CTG GCC GAT GAC GGT ATT GAG GTC TCC GCC GAC AAG ATC
 L   I   L   A   D   D   G   I   E   V   S   A   D   K   I
CAG ACC ATC CTC GGT GCC GCC AAG GTC CAG GAG GTT GAG CCC ATC
 Q   T   I   L   G   A   A   K   V   Q   E   V   E   P   I
TGG GCC ACC ATC TTC GCC AAG GCC CTC GAG GGT AAG GAC ATC AAG
 W   A   T   I   F   A   K   A   L   E   G   K   D   I   K
GAG ATC CTG ACC AAC GTC GGC TCC GCT GGT CCC GCC ACC GCC GGT
 E   I   L   T   N   V   G   S   A   G   P   A   T   A   G
GCC CCC GCC GCT GCT GGT GCC CGC GCT CCC GCT GAG GAG AAG AAG
 A   P   A   A   A   G   A   R   A   P   A   E   E   K   K
GAG GAG AAG GAA GAG GAG AAG GAG GAG TCC GAT GAG GAC ATG GGC
 E   E   K   E   E   E   K   E   E   S   D   E   D   M   G
TTC GGT CTC TTC GAC CAT CAT CAT CAT CAT CAT TAA GC
 F   G   L   F   D   H   H   H   H   H   H   *
```

Fig. 1. Nucleotide and amino acid sequence of (a) N- and (b) C-His_6 tagged (bold) Pen b 26. The additional residues to the native Pen b 26 are underlined.