Heterologous expression of Bromelain in *Escherichia coli*

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Abstract— **In spite of the fact that commercial bromelain supplements are available in the market, to date, none of them are produced and formulated from recombinant forms. They are extracted and purified (often partially) from the stem and fruit of pineapple. This makes the production of bromelain very difficult, less reliable, often contaminated and expensive. In this study, a recombinant bromelain from BL21 A clone was expressed as soluble and insoluble active enzyme. Maximum activity was observed at 4-hour post induction with 0.2% L-arabinose and over 60% of the enzyme was found to be expressed in soluble form. The enzyme fractions were purified using Nickel-NTA spin column. Purification fold and % yield of the purified lysate were found to be 35 and 75% respectively. SDS-PAGE results showed that the purified bromelain exhibited a single band with molecular weight of about 45kDa.**

Keywords-Bromelain, Ni-NTA, Ananas comosus, Luria Bertani

I. INTRODUCTION

The use of purified proteins for therapeutical purposes has been in existence for many decades. Hormones such as insulin and human growth hormones, and other proteins were extracted and purified from blood and other tissues before the emergence of recombinant DNA technology [1]. Enzymes constitute the largest portion of these purified proteins for industrial and therapeutic applications. Since the approval of insulin in 1982, more than 120 recombinant drug substances have been approved and become available as extremely valuable therapeutic options [2].

The name ''bromelain" was originally given to a mixture of proteases found in the juice of the stem and fruit of pineapple (*Ananas comosus*). Even now, bromelain is still used as collective name for the enzymes found in various members of *Bromeliacea*e family. The major peptidase present in the extracts of plant stem is called stem 'bromelain' while the major enzyme fraction found in the juice of pineapple fruit is named 'fruit bromelain'. Some other minor Cysteine endopeptidases (*ananain, comosain*) are also present in the pineapple stem bromelain.

Stem bromelain (E.C.3.4.22.32), belonging to papain family, is a glycosylated single chain protein containing of about 212 amino acid residues including seven Cysteines, one of which is involved in catalysis [3]. Pure stem bromelain is stable when stored at -20^{0}C and has optimum pH for activity at 6-8.5 for most of its substrates. The optimum temperature range for the enzyme is 50-60°C. It is mostly activated by cysteine while hydrogen sulphide and sodium cyanide are less effective [4]. However, heavy metals (like mercury and silver) and E-64 deactivate the enzyme.

Bromelain also has greater therapeutical applications. It was firstly introduced as a therapeutic compound in 1957 [5]. Bromelain actions include: (1) inhibition of platelet aggregation; (2) fibrinolytic activity;(3) anti-inflammatory action;(4) anti-tumour action; (5) modulation of cytokines and immunity; (6) skin debridement properties; (7) enhanced absorption of other drugs;(8) mucolytic properties; (9) digestive assistance ; (10) enhanced wound healing; and (11) cardiovascular circulatory improvement [5]. It is also used in baking industries and meat tenderization.

The use of conventional methods makes the production and formulation of bromelain very difficult, less reliable, often contaminated and expensive. Moreover, downstream processing is one of the major cost factors (up to 50%) in bioprocess. Hence, it is vital to develop efficient and cost effective processes that contribute towards the development of highly purified and cheaper bromelain. Consequently, this research work is geared towards the over expression of recombinant bromelain in *E .coli* using shake flasks and a 2-liter bioreactor and then purifying the enzyme using Nickel-NTA spin column chromatography.

II. MATERIALS AND METHODS

A. Chemical: All chemicals used were obtained from commercial sources and of highest purity.

B. Over expression of Recombinant Bromelain

E. coli cells harboring recombinant bromelain gene [6, 7] were grown over night in LB media (Luria Bertani) containing 100μg/ml ampicillin until the OD_{600nm} reached 0.6-1.0. The overnight culture was then diluted 100 fold in afresh LB media containing 100μg/ml ampicillin and was grown at 37 ^{o}C and agitated at 250 rpm in shake flasks and then a 2-L bioreactor up to a cell density of OD = 0.4. Inducer (L-arabinose) was then added to reach a final concentration

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of 0.2% and the induction continued for 4 hours. Cells were harvested from the spent media by centrifugation (8,000 rpm, 4 ^{o}C, and 20 min) and stored at -20°c until used [8].

C. Purification of the enzymes

The harvested cells were subjected to sonication on ice using 6- 10 second burst with 10 seconds interval at high amplitude. This was followed by centrifugation at 4°C, 12000 rpm for 30 minutes and supernatant was collected and purified by Nickel-NTA affinity chromatography. The enzymes were purified under native and denaturing conditions according to manufacturer's instructions.

D. Protein Determination

Proteins present in the enzyme preparations were determined by Bradford's method [9] using bovine serum albumin as standard.

E. SDS-PAGE Electrophoresis

After each step of purification, the protein fractions were tested by SDS-PAGE in 12.5% Polyacrylamide gels. Current was kept constant at 90V for 1 hour. Visualization was done by staining with Coomasie Brilliant blue R-250[10]

Table 1: Purification Table of native and denatured bromelain

Protein	**Vol. (ml)**	**Total protein (mg)**	**Activity (units)**	**Total activity (units x ml)**	**Specific activity (unit/mg)**	Purification fold	% Yield
Crude native	100	0.0184	0.556	55.6	30	1.0	100
Purified Native	50	0.0008	0.834	41.7	1039	35	75
Crude denatured	20	0.0852	0.214	4.28	2.5	1.0	100
Purified Denatured	10	0.0632	0.223	2.23	3.5	1.4	52

F. Measurement of Proteolytic Activity

The assay was carried out by the Silverstein's [11] method modified to reach optimal conditions of the enzyme. The activity was studied using N-a-Cbz-L-Gln-p-nitrophenyl ester as substrate. Assays were made at 37°C in 0.1 M Tris–HCl buffer (pH8.0) containing 25 mM cysteine and 1 mM of each substrate in the reaction mixture. Liberation of *p*-nitrophenol was followed spectrophotometrically at 405 nm using UV-visible spectroscopy system. An arbitrary enzyme activity unit (Ucbz) was defined as the amount of protease (g) that released one micromole of *p*-nitrophenolate per min in the assay conditions.

III. RESULTS AND DISCUSSION

The SDS-PAGE results of the bromelain are presented in Fig. 1. In (a) and (c), binding and elution efficiency of nickel-NTA spin column are depicted on native and denatured bromelain respectively. Single, homogenous band of bromelain, of molecular weight 45kDa was obtained in (a). However, multiple bands for denatured bromelain were obtained in (b). The unfolded proteins in (b) might have trapped some proteins during purification process. In (c), the purified native proteins showed homogenous single bands of 45kDa molecular weight.

From Table 1, it can be seen that the purification fold for both native and denatured bromelains are 35 and 1.4 while their corresponding % yield are 75 and 52 respectively. This implies that higher purification factor and yields were obtained in the native form of bromelain.

CONCLUSION

It can be concluded that the over expressed bromelain had over 60% of the protein as soluble form. In addition, higher yield and purification factor had been achieved in the production of native form of bromelain. The results also indicated that a homogenous, single native bromelain, of molecular weight of about 45kda has been produced and purified.

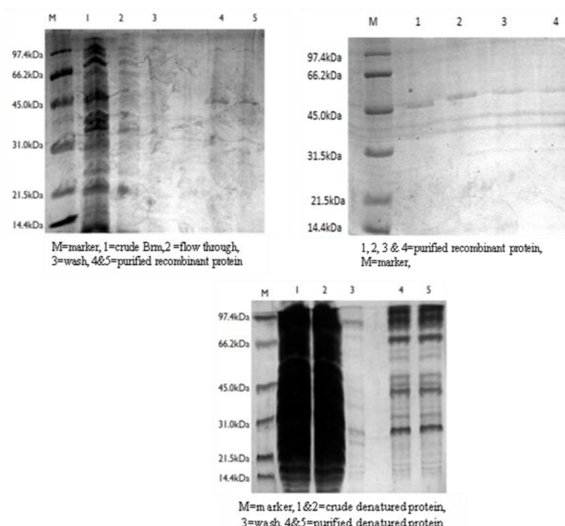

Fig 1: SDS-PAGE results for native and denatured bromelains

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