Journal of Chromatography B 1055–1056 (2017) 104–112

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Purification of β-mannanase derived from *Bacillus subtilis* ATCC 11774 using ionic liquid as adjuvant in aqueous two-phase system

Nur Fazrin Husna Abdul Aziz[a], Sahar Abbasiliasi[b], Hui Suan Ng[c], Pongsathon Phapugrangkul[d], Mohamad Hafizi Abu Bakar[a], Yew Joon Tam[e], Joo Shun Tan[a,*]

[a] Bioprocess Technology, School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia
[b] Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[c] Faculty of Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia
[d] Thailand Institute of Scientific and Technological Research, Bio-Sciences Department, 35, Moo3, Klong 5, Klong Luang, Pathumthani 12120, Thailand
[e] Laboratory of Immunotherapeutic and Vaccine Technology (LIVES), Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ARTICLE INFO

Keywords:
β-Mannanase
Aqueous two-phase system
Polyethylene glycol
Ionic liquid
Purification

ABSTRACT

The partitioning of β-mannanase derived from *Bacillus subtilis* ATCC 11774 in aqueous two-phase system (ATPS) was studied. The ATPS containing different molecular weight of polyethylene glycol (PEG) and types of salt were employed in this study. The PEG/salt composition for the partitioning of β-mannanase was optimized using response surface methodology. The study demonstrated that ATPS consists of 25% (w/w) of PEG 6000 and 12.52% (w/w) of potassium citrate is the optimum composition for the purification of β-mannanase with a purification fold (PF) of 2.28 and partition coefficient (K) of 1.14. The study on influences of pH and crude loading showed that ATPS with pH 8.0 and 1.5% (w/w) of crude loading gave highest PF of 3.1. To enhance the partitioning of β-mannanase, four ionic liquids namely 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF_4), 1-ethyl-3-methylimidazolium tetrafluoroborate ([Emim]BF_4), 1-butyl-3-methylimidazolium bromide ([Bmim]Br), 1-ethyl-3-methylimidazolium bromide ([Emim]Br) was added into the system as an adjuvant. The highest recovery yield (89.65%) was obtained with addition of 3% (w/w) of [Bmim]BF_4. The SDS-PAGE analysis revealed that the β-mannanase was successfully recovered in the top phase of ATPS with the molecular size of 36.7 kDa. Therefore, ATPS demonstrated a simple and efficient approach for recovery and purification of β-mannanase from fermentation broth in one single-step strategy.

1. Introduction

β-Mannanase is one of the major mannan-degrading enzymes from mannans. It has wide applications in biobleaching of pulp and paper, hydrolytic agent in detergent industry, hydrolysis of coffee extract, improvement of animal feed, use as fish feed additives, acts as slime control agent and use in pharmaceutical industry [1,2]. Mannanases could be produced by various sources of microorganisms. Among the bacteria, gram-positive bacteria, mainly *Bacillus* sp. show the mostly confined of degradation of mannan [3]. The *Bacillus* sp. which can produce high level of mannanase include *Bacillus subtilis* WY43 [4], *Baciilus amyloliquifaciens* [3] and *Bacillus circulans* [5].

Numerous purification strategies have been reported for mannanase all with varying degrees of success. The purification methods commonly employed include precipitations along with chromatographic techniques such as gel filtration, ion exchange and affinity chromatography. The major drawbacks of these conventional purification methods are associated with low yields and time intensiveness, expensive (non-economical), low throughput and complex scale-up [6] and the requirement for a skilled operator as such industries prefer purification processes that are less capital intensive, high yielding, rapid and robust with easy up-scaling procedure [7].

In recent years, ionic-liquid-based aqueous two-phase systems (IL-ATPS) have been object of a growing interest due to their potential in the design of novel separation processes. IL-ATPS as a new type of system in most separation processes have shown many advantages in separation and purification, such as system which is easy to be scaled up, faster mass transfer and balance, high extraction efficiency, low viscosity, little emulsion formation, no need of using volatile organic solvent, and gentle biocompatible environment. These novel ATPSs have been successfully used to separate *Bacillus cereus* cyclodextrin glycosyltransferase [8] and lipase by recombinant *E. coli* [9].

In view of the fact that IL-ATPS is an ideal purification technique for the separation, extraction and concentration of biomolecules, this study

* Corresponding author.
E-mail address: jooshun@usm.my (J.S. Tan).

http://dx.doi.org/10.1016/j.jchromb.2017.04.029
Received 18 October 2016; Received in revised form 9 March 2017; Accepted 14 April 2017
Available online 17 April 2017

investigated the purification of β-mannanase derived from *Bacillus subtilis* ATCC 11774 based on the ATPS which composed of ionic liquid and salt. In this respect, the effect of the molecular weight of polyethylene glycol (PEG), type of salt, pH of salt and crude enzyme loading in the partition efficiency of β-mannanase in ATPS were determined. RSM was used for optimization of the resources IL and maximizing the response of interests (*e.g* purification factor, yield). RSM is a great tool analysis in determination the optimum composition of ILs and salt able to minimizes the economic costly on purification mannanase on IL-ATPS with a better purification factor (P_F) and yield.

2. Material and methods

2.1. Materials

PEG with various average molecular weights (MW), ranging from 2000 to 10,000 (g/mol), were obtained from Sigma–Aldrich (St. Louis, MO, USA). 1-Ethyl-3-methylimidazolium tetrafluoroborate ($EmimBF_4$), 1-Ethyl-3-methylimidazodium bromide (EmimBr), 1-Butyl-3-methylimidazolium tetrafluoroborate ($BmimBF_4$), and 1-Butyl-3-methylimidazolium bromide (BmimBr) were obtained from Sigma Aldrich, USA. The protein assay kit and albumin standard were supplied by Bio-Rad, USA and Thermo Scientific Pierce, respectively. Luria Bertani (LB) broth, glucose and sodium chloride (NaCl) were sourced from Merck (Dramstadt, Germany). Sodium citrate ($C_6H_5Na_3O_7{\cdot}2H_2O$) and potassium phosphate were purchased from SAFC (St. Luois, MO, USA). All chemicals used in this study were of analytical grades except the ionic liquids which were of chemical grades.

2.2. Microorganisms and fermentation conditions

β-mannanase producing strain, *B. subtilis* ATCC 11774, was used in this study. A stock culture of *B. subtilis* ATCC 11774 was cultured at 37 °C and kept in 40% (v/v) glycerol aseptically at −20 °C. An inoculum was prepared by adding 10% (v/v) of stock culture into 10 mL of Luria broth (LB) in 50 mL conical flask and incubated in an incubator shaker at 30 °C, agitated at 200 rpm (Orbital Incubator Shaker, Infors, Switzerland) for 12 h.

2.3. Cultivation of B. subtilis for β-mannanase production

A 10% (v/v) of inoculum was added into 100 mL of LB medium containing 10%(v/v) of banana waste juice and 1%(w/v) of locust bean gum as carbon source in a 250 mL shake flask. The culture was incubated at 30 °C in incubator shaker and agitated at 200 rpm for 24 h prior to harvesting. The cultures were centrifuged at 4000 for 30 min at 4 °C (rotor model 1189, Universal 22R centrifuge, Hettich AG, Switzerland). The supernatant that contained crude β-mannanase enzyme was collected and kept under −20 °C in prior to use.

2.4. Construction of binodal curve

The binodal curves were constructed using turbidometric titration technique as described by Hatti-Kaul [10]. Three types of PEG with different molecular weight (PEG 2000, 4000 and 6000 g/mol) were used in this step. For each PEG, a series of known total composition and weight are prepared. The system was added with diluent drop by drop until the mixture turns clear. This indicated that one phase system has been formed. The amount of diluent added were measured and recorded. The final weight percentage of the PEG and the potassium citrate which indicated the critical points on the binodal curve, were calculated. The critical points were plotted to construct a binodal curve on a phase diagram.

2.5. ATPS partition experiments

ATPS was prepared from PEG stock solutions [(50% w/w) of different MWs (PEG 2000, 4000 and 6000 g/mol)] and salt stock solutions [(40% w/w) of potassium phosphate, potassium citrate and sodium acetate]. The phase systems were prepared in 15 mL centrifuge tubes. PEG stock solution, salts stock solution at pH of 7 and 1.25% (w/w) of crude samples of known masses were weighed into the centrifuge tubes. Appropriate mass of distilled water were then added into the system in order to obtain a final mass of 5 g system. The feed solutions were stirred thoroughly using a vortex mixer and then centrifuged at 4000 × *g* for 10 min to complete the phase separations. Finally, the volumes of both phases were measured and samples from coexisting phases were obtained for β-mannanase activity and protein assays. All separation processes were performed at room temperature.

2.6. Selection of phase component for stability of β-mannanase activity

The suitable of top and bottom phase component of the systems were determined prior to ATPS partitioning experiment. For the determination of a suitable top phase, three different molecular weights of PEG which are PEG 2000, PEG 4000 and PEG 6000 were used in this experiment. For the determination of suitable bottom phase, three types of salts which are potassium phosphate, potassium citrate and sodium acetate were used in this experiment. All PEGs or salts were weighed in 15 mL Falcon tubes at different weight percentage which were 5% (w/w), 10% (w/w), 15% (w/w) and 20% (w/w). One gram of crude enzymes was added into each of the systems before the systems are being toped up with distilled water to make a total weight of 5 g system. The systems were left at room temperature for 6 h and subjected to β-mannanase activity analysis to determine the stability of β-mannanase activity in different phase components.

2.7. Optimization of separation parameters

Based on prior selection of suitable phase component, highest activity of β-mannanase was obtained in potassium citrate. The interaction effect of PEG (top phase of this system) and potassium citrate at pH 8.0 (bottom phase of the system) was then optimized using response surface methodology (RSM). A five-level, full-factorial central composite design (CCD) with two independent variables, that is, composition of PEG and potassium citrate was applied in this study (Table 1).

Design Expert (version 7.1.6, Stat-Ease Inc., Minneapolis, MN, USA) was used for regression modeling and data interpretation. The observed responses from CCD design were then fitted to the following polynomial Eq. (1) as shown by

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i \beta_{ii} x_j + \varepsilon \quad (1)$$

where Y is the predicted response; *i* and *j* are the index numbers for the pattern; *β* is the offset term; *βi*, *βii*, and *βij* are the coefficients for the linear, quadratic, and interaction effects, respectively; *xi* and *xj* are the coded variables; and *ε* is the error. The regression equation was optimized by an iterative method to achieve the optimum values. In this step, the molecular weight of PEG, composition of PEG and

Table 1
Factors and levels for five level full factorial design of β-mannanase partitioning.

Factors	Range and levels				
	−α	−1	0	+1	+α
PEG concentration (% w/w)	12.93	15.00	20.00	25.00	27.07
Potassium citrate concentration (% w/w)	8.96	10.00	12.50	15.00	16.04