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Recovery of Bacillus cereus cyclodextrin glycosyltransferase and recycling of phase components in an aqueous two-phase system using thermo-separating polymer

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Introduction

Aqueous two phase system (ATPS) is generally defined as formation of two immiscible phases which consist of incompatible polymers or polymer together with inorganic salts [1]. ATPS has been widely applied in many purification and separation processes of biomaterials such as cells, cell organelles, proteins and nucleic acids due to its high water concentration in both phases [about 70–90% (w/w)], low interfacial tension, non-toxic, inflammable properties which provides a mild condition in large scale purification process [2–5]. Besides, ATPS is practically environmental friendly in nature as an extraction media. Different combinations of salt and polymer or polymer and polymer ATPSs have been performed in various studies to date [6]. The conventional ATPSs constructed by polyethylene glycol (PEG)/salts and PEG/dextran are the most commonly developed ATPSs [7]. Formation of PEG/salt ATPS is caused by the salting out of polymer by salt whereas PEG/dextran ATPS is formed due to the incompatibility of both polymers. The drawbacks of these ATPSs are the complexity of separating the phase-forming polymers from target enzymes and the high cost of phase-forming polymers (e.g. Dextran) for large scale operations [8,9]. To improve the ATPS established previously, a more economical and environmental friendly ATPS with the ability to retain the biological activity of enzymes is preferable as compared to other conventional ATPSs. As such, the recycling of the phase components by temperature induced phase separation has caught the attention of researchers because the overall cost can be minimized and the process of separating the target protein from phase solutions will be simplified. However, information regarding this type of ATPS is still limited [4,8,10–12].

Recently, the thermo-separating phase-forming polymer (i.e. EOPO) has been introduced to overcome the drawbacks encountered by the traditional ATPSs (e.g. PEG/salts and PEG/dextran) [13–15]. In these ATPSs, a primary two-phase system is first formed and the target protein is partitioned to the EOPO top phase. Next, the EOPO phase is withdrawn and heated above the cloud point of the polymers to induce thermo-separation and a new two-phase system consisted of a water top phase and a polymer bottom phase is observed. Target protein is harvested from water phase whereas the EOPO polymer can be recovered from EOPO bottom phase of this particular system. EOPO random

copolymers (linear and non-ionic) can be separated from aqueous solution by heating the solution above a cloud point (or known as lower critical solution temperature, LCST), which is at low temperature of around 50 _C [3,13–16]. In this case, the polymers could be recycled and the salt component could also be reused in subsequent ATPS extraction [11,14,15]. Different EO and PO contents in the EOPO random copolymers exhibit different LCST and as the PO content increases, the LCST of the copolymers decreases. In addition, LCST of an EOPO random copolymer can be reduced by addition of neutral salts such as sodium sulfate. High molecular weight of random EOPO polymers gives a more efficient temperature induced phase separation and recovery. PEG is also able to be thermo-induced and separated from the target protein. However, it is not an economical approach to recycle PEG because LCST for PEG (in the range of 95–180 _C, depending on the PEG molecular weight) is higher as compared to that for EOPO [16].

Cyclodextrin glycosyltransferase (CGTase; E.C. 2.4.1.19) is an extracellular enzyme that is capable of converting starch into cyclic compounds namely cyclodextrins (CDs) which is highly demanded in pharmaceutical and biotechnology field via cyclization reaction [17–21]. Species of Bacillus is reported as the most common producers of CGTase [22–25]. In this study, the production of CGTase was carried out by fermentation using Bacillus cereus [17]. The recovery of lipase derived from Burkodelderia cenocepacia strain ST8 and recycling of phase components (i.e. EOPO and salt) in ATPS has been studied in a previous publication [26]. However, the application of such a recyclable ATPS in the processing of B. cereus feedstock has not yet been investigated. Therefore, the previous work of Show et al. [26] was extended in order to investigate the feasibility of such an ATPS (i.e. EOPO and potassium phosphate) for the recovery of CGTase from B. cereus feedstock. The effects of tie-line length (TLL), volume ratio and pH upon CGTase recovery performance were studied.

2. Materials and methods

2.1. Materials

The chemicals, cyclodextrins (CDs), poly(ethylene glycol-ranpropylene glycol) with molecular weight of 2500 and 12,000, poly(ethylene glycol-ran-propylene glycol) monobutyl ether with molecular weight of 970 and 3900 and bicinchoninic acid solution were purchased from Sigma–Aldrich Co. (St. Loius, MO, USA). Phenolphthalein, di-potassium hydrogen phosphate (K_2HPO_4) and potassium di-hydrogen phosphate (KH_2PO_4) were sourced from Merck (Darmstadt, Germany). All other chemicals applied were analytical grade.

2.2. Bacterial strain and culture conditions for CGTase production

The bacterial strain and culture conditions for CGTase production have been described previously [17].

2.3. CGTase activity

The CGTase activity measurement method has been described previously [17,27,28].

2.4. Acetone precipitation

Acetone precipitation step with modification [29,30] was employed prior to BCA assays to reduce the interferences of the thermo-separating polymers on the samples. A 100 ll sample was added into 400 ll of acetone in a 500 ll Eppendorf tube. The mixture was vortexed to obtain a homogeneous solution and then incubated at _30 _C for a minimum of 1 h. After the incubation, the tube was centrifuged at 12000 rpm and at 4 _C for 15 min. Next, the supernatant was removed and 100 ll of deionized water was added into the precipitated protein pellet. The protein pellet was re-suspended and redissolved in the deionised water to obtain a protein solution. This protein solution was subsequently employed for BCA assay.