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A protease-resistant α-galactosidase from *Pleurotus djamor* with broad pH stability and good hydrolytic activity toward raffinose family oligosaccharides

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ABSTRACT

An acidic α-galactosidase designated as PDGI (*Pleurotus djamor* α-galactosidase) was purified to homogeneity with 290-fold purification and a specific activity of 52.18 units/mg by means of ion exchange chromatography and gel filtration chromatography. PDGI is a monomeric protein exhibiting a molecular mass of 60 kDa in SDS-PAGE and gel filtration. The optimum pH and temperature of the enzyme with pNPGal as substrate were 5.0 and 53.5 °C, respectively. It displayed great pH stability within the pH range 3.0–10.0. Besides, the enzyme harbored remarkable resistance to acid protease and varying degrees of tolerance to other proteases: trypsin > collagenase Type-I > α-chymotrypsin neutral protease > proteinase K. It was strongly inhibited by K^+, Cd^{2+}, Cu^{2+}, Hg^{2+}, Al^{3+}, Fe^{3+} and Ag^+ ions. The chemical modification reagents diethypyrocarbonate (DEPC), 2,3-butanedione (DIC) and trinitrophenol (TNBS) increased the activity of PDGI 1.5-fold whereas *N*-bromosuccinimide (NBS) and parachloro-mercuri-benzoate (PCMB) drastically suppressed its activity. PDGI displayed activity toward stachyose and raffinose. The Km values for hydrolysis of pNPGal, stachyose and raffinose were 0.76, 7.63 and 6.29 mM, respectively. Furthermore, PDGI degraded raffinose and sthachyose. These results suggest that PDGI has great potential for elimination of the non-digestible and flatulence-causing oligosaccharides stachyose and raffinose from legumes.

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1. Introduction

α-Galactosidases (melibiase; α-D-galactoside galactohydrolases; EC 3.2.1.22), which are widely distributed in plants, animals and microorganisms, catalyse the hydrolysis of α-1,6-linked α-galactopyranosyl residues from various galacto-oligosaccharides and polymeric galactomannans [1]. α-galactosidases have various applications in sugar-production [2], pulp and paper [3], food and feed additives [4–7], blood group transformation, treatment of Fabry's disease, and xenotransplantation [8–11]. Despite their hydrolytic activity, some α-galactosidases can also be applied to the synthesis of α-galactosides by transglycosylation and reverse hydrolysis reactions [12,13]. *Penicillium oxalicum* α-galactosidase exhibited a high rate of transglycosylation in the reaction with melibiose [14]. Due to the release of galactosyl residues from raffinose which hold back sucrose crystallization, α-galactosidase is also used to improve the yield of sucrose in the production of the beet sugar [2].

Because of indigestibility and induction of flatulence in monogastric animals, α-D-galactosides, mainly raffinose and stachyose, are identified as antinutritional factors in soybean and other legume seeds. In the past few years, microbial α-galactosidases have been extensively added to the feed additives to degrade raffinose-family oligosaccharides(RFOs), in order to improve efficiency and nutritional value [4,15–17].

Edible fungi have been used as functional foods or drugs more and more often. It has great advantages as the food source of α-galactosidases compared with industrial enzymes taking into account the safety considerations and fewer adverse effects. *Pleuro-*

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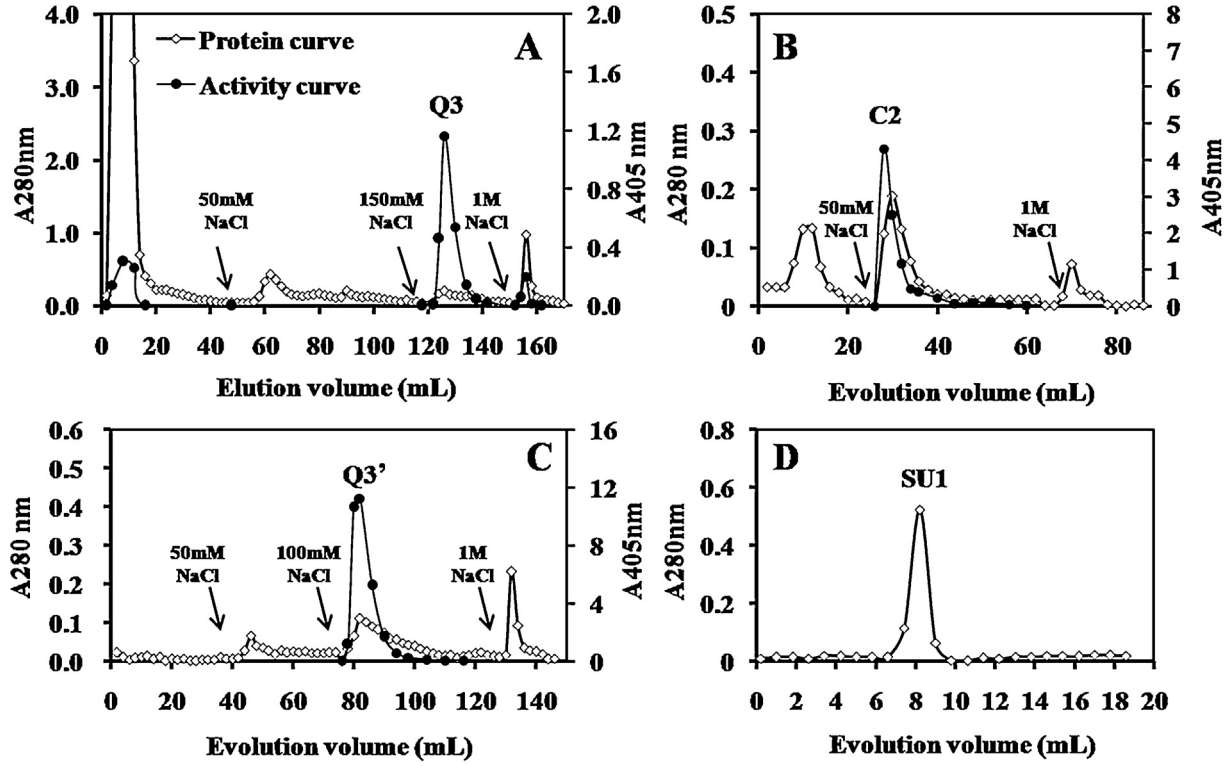

Fig. 1. Profile of elution of PDGI from a (A) Q- Sepharose column, (B) CM-cellulose column, (C) Q- Sepharose column and (D) Superdex 75 HR 10/30 gel filtration column.

tus djamor is a favorite edible mushroom known for its bright pink color and unique flavor. Up till now, very little research on it has been reported. Salmones et al. studied the relationship between its mycelial growth rate and the production of basidiomata [18]. Mata and Pérez-Merlo investigated its spawn viability after freezing in liquid nitrogen without a cryoprotectant [19]. Salmones et al. compared its biomass production and substrate biodegradation of cultivation on coffee pulp and wheat straw [20]. James et al. analyzed its genetic structure and diversity of the A and B mating-type genes [21]. Kalmiş et al. evaluated its ability to decolorize Benazol Black ZN textile dye [22]. Wu et al. isolated a novel ribonuclease from its fruiting bodies [23]. Moreover, Borges et al. extracted an extracellular polysaccharide from its fruiting bodies and studied its antitumor activity [24]. Mishra et al. evaluated its antioxidant properties [25]. However, no studies have appeared concerning the isolation and characterization of α-galactosidases from *P. djamor*.

In view of the various physiological functions and high-activity of α-galactosidases, herein we report the purification and enzymatic properties of an α-galactosidase (defined as PDGI) from the fruiting bodies of *P. djamor*. The ability of PDGI to hydrolyse various natural substrates, especially the RFOs, was also assessed in order to explore its potent applications in the food and feed industries.

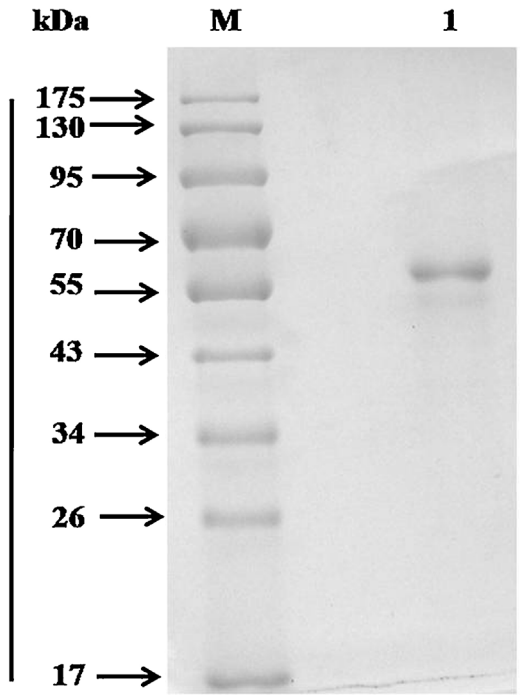

Fig. 2. SDS-PAGE analyses of PDGI. Lanes: M, molecular mass standards; 1, Superdex 75 fraction.

2. Materials and methods

2.1. Plant materials and chemicals

Fresh fruiting bodies of *P. djamor* were acquired from Tongzhou in Beijing. Q-Sepharose and CM-cellulose were purchased from Sigma Chemical Co., USA. Superdex G-75 HR 10/30 and AKTA Purifier were obtained from GE Healthcare, USA. The substrates 4-nitro-phenyl α-D-galactopyranoside (pNPGal), *o*-nitrophenyl α-D-galactoside (oNPαGal), 4-nitrophenyl β-D-glucuronide, locust bean gum, guar gum, melibiose, galactose, lactose, sucrose, glucose, xylose, fructose, stachyose and raffinose were purchased from Sigma Chemical Company (St. Louis, MO, USA). Acid protease, neutral protease, proteinase K, collagenase type-I, α-chymotrypsin, subtilisin and trypsin were purchased from Sigma Chemical Company. Other chemical reagents used were of analytical grade.

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