Food Chemistry 198 (2016) 119–124

Contents lists available at ScienceDirect

Food Chemistry

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

Novel inexpensive fungi proteases: Production by solid state fermentation and characterization

Paula Kern Novelli [a,*], Margarida Maria Barros [b], Luciana Francisco Fleuri [c]

[a] FMVZ, UNESP (University of São Paulo State), Department of Chemistry and Biochemistry, IB, Botucatu, SP, Brazil
[b] FMVZ, UNESP, Department of Animal Breeding and Nutrition, Botucatu, SP, Brazil
[c] Laboratory of Bioprocess, Department of Chemistry and Biochemistry, IB, UNESP, Botucatu, SP, Brazil

ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 16 October 2015
Accepted 16 November 2015
Available online 17 November 2015

Keywords:
Fungal protease
Agro-industrial residues
Solid state fermentation
Biochemical characteristics

ABSTRACT

A comparative study was carried out for proteases production using agroindustrial residues as substrate for solid state fermentation (SSF) of several fungal strains. High protease production was observed for most of the microorganisms studied, as well as very different biochemical characteristics, including activities at specific temperatures and a wide range of pH values. The enzymes produced were very different regarding optimum pH and they showed stability at 50 °C. *Aspergillus oryzae* showed stability at all pH values studied. *Penicillium roquefortii* and *Aspergillus flavipes* presented optimum activity at temperatures of 50 °C and 90 °C, respectively. Lyophilized protease from *A. oryzae* reached 1251.60 U/g and yield of 155010.66 U/kg of substrate. Therefore, the substrate as well as the microorganism strain can modify the biochemical character of the enzyme produced. The high protease activity and stability established plus the low cost of substrates, make these fungal proteases potential alternatives for the biotechnological industry.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Proteases (EC 3.4.23.18) are one of most important and abundant enzymes produced by the biotechnology industry, consisting of more than 25% of biomolecules produced for industrial application and 60% of the whole enzyme market (Kumar, Sahai, & Bisaria, 2012; Ramakrishna, Rajasekhar, & Reddy, 2010). Proteases contribute to several chemical and biochemical reactions that take place in the food, beverage, pharmaceutical, cosmetics, and other industries, hydrolyzing peptide bonds in proteins and polypeptides (Uyar & Baysal, 2004). In the animal food industry, where protein-based food is often characterized by the presence of several anti-nutritional peptides and low nutritional availability, proteases can be particularly helpful by improving protein digestibility, as well as reducing environmental pollution (Pariza & Cook, 2010).

Proteases can be obtained from several sources, including plants, animals, and microorganisms. Even though a wide variety of microbial proteases are available, use of these enzymes on an industrial scale is still limited by their high production costs and the fact that their activity is often limited to a restricted range of biochemical characteristics (Akhtar et al., 2013). Therefore, studies involving other microbial enzyme sources are necessary.

Although most microbial enzymes are produced by submerged fermentation, solid state fermentation (SSF) using agro-industrial residues allows the production of these biomolecules at a lower cost and it is environmentally friendly (Salihu, Alama, Karim, & Salleh, 2012). SSF is also a cost-effective process as it uses agroindustrial wastes, like seeds, peels, husks, bark, and bran to produce valuable bioactive molecules.

Another important advantage of SSF is the higher growth rate exhibited by fungi on solid substrate as compared to submerged fermentation; the morphology of filamentous fungi allows them to colonize the substrate surface and matrix in search of nutrients, consequently secreting higher levels of metabolites and enzymes (Barrios-González, 2012). Filamentous fungi of industrial interest include several species of *Aspergillus* sp. (Van Der Hombergh, Van de Vondervoortb, Fraissinet-Tachetb, & Visserb, 1997), as they are capable of substrate adaptation and produce several metabolites with high biological activities. The genus A*spergillus* is also considered non-toxic, recognized as a safe microorganism by the Food and Drug Administration (FDA), denominated Generally Recognized as Safe (GRAS), and used for human and animal nutrition (Gotou, Shinoda, Mizuno, & Yamamoto, 2009; Morita et al., 2010; Vishwanatha, Rao, & Singh, 2009).

* Corresponding author at: Departamento de Química e Bioquímica, Instituto de Biociências de Botucatu, UNESP – Campus de Botucatu, Prof. Dr. Antonio Celso Wagner Zanin, S/N, Distrito de Rubião Júnior, PO Box 510, ZIP code: 18618-689, Botucatu/SP, Brazil.

E-mail addresses: pnovelli@hotmail.com (P.K. Novelli), mbarros@fmvz.unesp.br (M.M. Barros), Luciana@ibb.unesp.br (L.F. Fleuri).

http://dx.doi.org/10.1016/j.foodchem.2015.11.089
0308-8146/© 2015 Elsevier Ltd. All rights reserved.

Some studies have pointed out the promising enzyme production by SSF with different organic residues such as soybean meal, wheat and rice, orange peels, apple and banana (Karatas, Uyar, Tolan, & Baysal, 2013;. Chutmanop, Chuichulcherm, Chist, & Sirinophakun, 2008; Monton, Unrean, Pimsamarn, Kitsubun, & Tongta, 2013). Therefore, the solid fermentation allows reducing the value of the final product and, yet, can be considered environmentally friendly as it uses low cost raw materials rejected in the agricultural industry during their production process. In the Brazilian scenario, which is heavily focused on agriculture economy; especially soy, wheat, corn and sugarcane, the technological potential for efficient reuse of agro-industrial waste can contribute to the development of high added value products such as enzymes, organic acids, flavors and fragrances, pigments, polysaccharides and hormones, adding value to this residue produced in large quantities, (Soccol & Vandenberghe, 2003). These biotechnological processes, especially SSF, can be applied to reduce costs and enable the use of enzymes for human and animal consumption.

In this study we evaluated the production of inexpensive proteases from several GRAS fungal strains using agroindustrial waste, wheat bran and soybean bran. The different residues used as substrate can modify fungi metabolic expression and produce enzymes with distinct characteristics which can be applied in the biotechnological industry in many ways. The combination of the microbial cell and different substrates comprises metabolic and biotransformation process that can generate several cellular products. Additionally, the microorganism and substrate selection plus the biochemical characteristic of the enzyme produced are important factors to evaluate its biotechnological potential and target the possible applications for industrial processes (Castro & Sato, 2013). Moreover, because the biochemical characterization of enzymes indicates biocatalyst performance and can predict the successful use of the enzyme for specific applications (Couto & Sanromán, 2006; Okino-Delgado & Fleuri, 2014), we also determined optimum and stability pH and temperature for these proteases.

2. Materials and methods

2.1. Microorganisms

Fungal samples from lyophilized strains *Aspergillus niger* (INCQS 40018), *A. niger* (INCQS 40065), *Aspergillus flavipes* (INCQS 40024), *Aspergillus brasiliensis* (INCQS 40036), *Aspergillus oryzae* (INCQS 40068), and *Penicillium roquefortii* (INCQS 40074) from Osvaldo Cruz Institute (FIOCRUZ) were inoculated in Potato Dextrose Agar (PDA) and incubated at 30 °C observing the growth period for each strain, which range between 120 and 192 h. They were maintained at 4 °C in PDA under a sterile layer of Vaseline and sub-cultured every three months. These strains together with 10 more fungi strains from the Bioprocess Laboratory, Department of Chemistry and Biochemistry, Institute of Biosciences of Botucatu (UNESP) were tested for enzyme production. Total of 8 strains, coded as 13, 51, 58, 68, 77, 129, 159, and 162, and two already identified as *A. niger* (01 and 40015) were selected for tests and screening of protease production based on previous studies for another enzymes production (Fleuri et al., 2014).

Fungi selected for this study are considered non-toxic and safe microorganisms, designated GRAS, and therefore can be used for human and animal nutrition.

2.2. Enzyme production

The proteases were obtained by SSF using two different substrates provided by the Department of Animal Breeding and Nutrition Feed Mill, University of São Paulo State: (1) wheat bran and (2) soybean bran. The culture medium was composed of 10 g of substrate (dry weight) and the moisture content was adjusted to 50% (w/v), substrate and sterilized water. Erlenmeyer flasks (250 mL) containing the culture medium were sterilized for 15 min at 121 °C under 1 atm of pressure, then subsequently inoculated with 2 mL of fungi suspension (10^7 spores/mL), followed by incubation at 30 °C for 120 h, according Castro and Sato (2013) with modifications. After incubation, 50 mL of distilled water was added to each flask. The culture medium was mixed manually using a glass rod, then left to stand for 1 h with occasional stirring. Enzyme extracts were collected in single flasks by filtration through cheesecloth. The crude extract enzyme activities were determined as described below.

The scale up of the process was done increasing the number of Erlenmeyer flasks and, at the same time, using flasks with double the size maintaining the proportion of substrate and sterilized water.

2.3. Protease activity

The protease activity was measured using azocasein as substrate, with modifications (Charney & Tomarelli, 1947). The reaction media were 0.5 mL of azocasein 0.5% in sodium phosphate buffer pH 7.0 (0.1 M) and 0.5 mL of crude enzyme extract, which were incubated for 40 min at 37 °C. The reaction was stopped with 0.5 mL trichloroacetic acid (TCA) 10% and centrifuged at 4677×*g* for 15 min at 15 °C; 1 mL of potassium hydroxide (5 M) was added to 1 mL of the filtrate. The protease activity unit was defined as the quantity of enzyme necessary to increase 0.1 of absorbance at 428 nm in the assay conditions.

2.4. Enzymatic kinetics

Maximum enzyme activity kinetics of selected fungi strains in wheat bran and soybean bran were performed at different fermentation times (from 72 to 168 h) by SSF under the conditions established above in the enzyme production.

2.5. Biochemical characterization

For biochemical characterization, the optimal activity and stability of enzymes at different pH and temperature were tested using enzymes produced by SSF in both substrates at the best fermentation time according production kinetics above.

2.5.1. Effect of temperature on activity and stability

The optimum temperature was determined as described for each enzyme activity (pH 7.0 to protease) at different temperatures, as follows: 20, 30, 40, 50, 60, 70, 80, and 90 °C.

The temperature stability was determined by incubating the crude enzyme extract at different temperatures for 1 h, followed by determining the residual activity as described in the section above for protease activity.

2.5.2. Effect of pH on activity and stability

Optimum pH was determined as described for each enzyme activity (37 °C) using buffer solutions at different pH values as follows: 0.1 M acetate buffer pH 4.0 and 5.0; 0.1 M sodium phosphate buffer pH 6.0 and 7.0; and 0.1 M borax–boric acid buffer pH 8.0 and 9.0.

The pH stability was determined by incubating the crude enzyme extract in buffer solutions at different pH values at 30 °C for 24 h, followed by determining the residual activity as described in the section on protease activity.

Download English Version:

https://daneshyari.com/en/article/1184783

Download Persian Version:

https://daneshyari.com/article/1184783

Daneshyari.com