Bioresource Technology 222 (2016) 210–216

Contents lists available at ScienceDirect

Bioresource Technology

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

Simultaneous production of proteases and antioxidant compounds from agro-industrial by-products

Ailton Cesar Lemes [a], Gabriel Teixeira Álvares [a], Mariana Buranelo Egea [b], Adriano Brandelli [c], Susana Juliano Kalil [a,*]

[a] Universidade Federal do Rio Grande, Escola de Química e Alimentos, Rio Grande, RS 96201-900, Brazil
[b] Instituto Federal Goiano – Campus Rio Verde, Rio Verde, GO 75901-970, Brazil
[c] Universidade Federal do Rio Grande do Sul, Instituto de Ciência e Tecnologia dos Alimentos, Departamento de Ciências dos Alimentos, Porto Alegre, RS 91501-970, Brazil

HIGHLIGHTS

- Different bioproducts was obtained simultaneously using different by-products.
- Integrated process was enabled to obtain proteolytic and keratinolytic enzymes.
- Residual biomass provided the highest protease activity (1306.6 U/mL) at 32-h.
- The reused feather meal reached the highest keratinolytic activity (89 U/mL) at 32-h.
- Hydrolysates produced in cultivation have great potential as natural antioxidants.

ARTICLE INFO

Article history:
Received 20 June 2016
Received in revised form 30 September 2016
Accepted 1 October 2016
Available online 3 October 2016

Keywords:
Bacillus
Feather meal
Biomass
Keratinase
Integrated processes

ABSTRACT

The use of processes for simultaneous production of bioproducts as enzymes and bioactive compounds is an interesting alternative to reduce environmental impacts. Thus, the aim of this study was to produce simultaneously, using the biorefinery concept, both proteases and bioactive compounds with antioxidant activity from *Bacillus* sp. P45 cultivation by using different by-products. The integrated process developed in this study enabled to obtain enzymes with proteolytic and keratinolytic properties in a process with alternate substrates from agro-industrial by-products (feather meal, residual feather meal and biomass), thus, creating an interesting alternative to managing them. The residual biomass provided the highest protease activity (1306.6 U/mL) and the reused feather meal reached the highest keratinolytic activity (89 U/mL), both at 32 h of cultivation. Moreover, hydrolysates produced in cultivation using feather meal and residual biomass had high antioxidant activity, they have great potential as natural antioxidants.

1. Introduction

Development of bio-sustainable and renewable resource technologies is extremely important on environmental contexts. Thus, the use of agro-industrial waste involves the management of materials generated as by-products to prevent environmental pollution. Waste can contain many valuable substances and, through a suitable process or technology, this material can be converted into value-added products or raw materials that can be used in secondary processes (Lemes et al., 2016a).

To reduce the impact of wastes, the biorefinery concept has increasingly focused on obtaining products of commercial significance from low value residual agro-industrial by-products (Kiskini et al., 2016). The biorefinery concept aims to optimize the yield of a range of components rather than to maximize the yield of a single component. Current strategies for by-products conversion to useful products are based on individual production chains. However, different bio-based industries can either combine or couple their material flows so that the residue from one industry becomes an input for another industry. This will result in optimal utilization of all components for generation of multiple products in an integrated biorefinery system with economic and environmental perspectives (Cherubini, 2010; FitzPatrick et al., 2010).

The biorefinery concept can be applied to produce simultaneously different bioproducts as the protease enzyme and bioactive compound with antioxidant activity using different waste of different biotechnological processes.

* Corresponding author.
E-mail address: dqmsjk@furg.br (S.J. Kalil).

http://dx.doi.org/10.1016/j.biortech.2016.10.001
0960-8524/

Among these residues are highlighted the use of non-solubilized feather meal resulting from biotechnological process of protease production by *Bacillus* sp. P45 (Daroit et al., 2011a) and the residual biomass generated in the production and extraction of β-galactosidase enzyme (Lemes et al., 2012), both with high protein contents. Instead of being discarded, these wastes can be used for producing enzymes and bioactive compounds with antioxidant activity.

Microbial proteases can be produced by *Bacillus* sp. P45, which was isolated from the intestine of *Jaraqui* (*Piaractus mesopotamicus*), a fish found in the Amazon basin, in the north of Brazil. Previous characterization, based on 16S rDNA sequencing, showed that this strain which clustered with the *Bacillus subtilis* group (Sirtori et al., 2006), a microorganism generally recognized as safe (GRAS), can be used in the production of food and pharmaceuticals. These enzymes, which have shown great potential for protein hydrolysis (Daroit et al., 2009; Sirtori et al., 2006), can be useful in enzymatic milk-clotting processes (Daroit et al., 2012; Lemes et al., 2016b). Furthermore, some characteristics indicate their potential use in the food industry, e.g., in the tenderizing meat process, bakeries and breweries, besides in pharmaceutical products, as additives in detergent and textile industry and in the process of hair removal and leather processing (Vijayaraghavan et al., 2014).

Additionally, compounds with functional properties, such as substances with antioxidant activity, can be generated throughout microbial cultivation. Antioxidant compounds have received significant attention not only because they protect the human body against oxidative stress, but also because they prevent a range of chronic degenerative diseases (Kaur and Kapoor, 2001). Furthermore, these compounds have gained greater prominence because of some synthetic antioxidants such as 2-tert-butyl-4-hydroxyanisole (BHA) and 2,6-di-tert-butyl-4-methylphenol (BHT), commonly used for slowing down oxidative processes in food and biological systems. Since they can pose risks to human health, their use has been restricted to food additives (Barlow and Schlatter, 2010). In the current literature, some reports describe the possible toxicity of these compounds in the human body, a fact that has resulted in the search for alternative sources of safe and effective antioxidants (Espín et al., 2007).

Diverse agro-industrial wastes have been investigated as substrates for biotechnological processes (Abd-Elhalem et al., 2015; Gudina et al., 2016; Maiti et al., 2016; Neethu et al., 2015; Tang et al., 2015). However, no previous reports have been published on the production of enzymes with biotechnological interest from agro-industrial by-products in integrated and consecutive use of substrates in culture medium, resulting in less environmental impact due to use of the generated waste. Therefore, the aim of this study was to develop an integrated and innovative biotechnological process to obtain enzymes and bioactive compounds from agro-industrial waste and by-products.

2. Materials and methods

Protein substrates, used in *Bacillus* sp. P45 cultivation to yield protease and bioactive compounds with antioxidant activity, were obtained according to the flowchart shown in Fig. 1. The process aimed the use of by-products generated by integrated biotechnological processes.

2.1. Obtaining protein substrates for protease production

The feather meal (Substrate 1) was produced from chicken feathers. Feathers were washed in water and autoclaved at 121 °C for 30 min. After the feathers were dried at 50 °C for 96 h (humidity ⩽10.0%) and powdered by a knife mill to a particle size ⩽0.5 mm.

Reused feather meal (Substrate 2) was obtained at the end of *Bacillus* sp. P45 cultivation with previously produced feather meal (Substrate 1). The washed feathers were drying at 50 °C for 96 h (humidity ⩽10.0%) and triturated by a knife mill to a particle size ⩽0.5 mm.

The residual biomass (Substrate 3) was obtained from the extraction process of the β-galactosidase enzyme from *Kluyveromyces marxianus* CCT 7082 (Lemes et al., 2012) using a ultrasonic homogenizer for cells disruption for 30 min. The disrupted cell was washed, centrifuged and dried at 50 °C for 96 h. Subsequently, the dry biomass was macerated and standardized as to its grain size (particles ⩽0.125 mm).

Substrate 4 was composed of a mixture of 50% feather meal (Substrate 1) and 50% residual biomass (Substrate 3). All substrates were characterized in relation to protein and moisture content in order to get equal proportions of protein in the final composition of the medium, as described below.

2.2. Protease and bioactive compounds production

The enzyme and the compounds with antioxidant activity were produced by submerged cultivation of *Bacillus* sp. P45 (GenBank accession number AY962474). It was kept in brain-heart agar (BHA) at 4 °C. For inoculum preparation, this strain was grown on BHA at 30 °C for 24 h. Cultures were scraped from the agar surface, added to a sterile 8.5 g/L NaCl solution and mixed until a homogeneous suspension with $O.D._{600}$ of 0.5 was obtained (Daroit et al., 2011a).

The enzyme and the compounds with antioxidant activity were produced by submerged cultivation, as described by Daroit et al. (2011a), in an optimized mineral medium composed of (g/L): NaCl (0.5), KH_2PO_4 (0.4), K_2HPO_4 (0.3), NH_4Cl (1.9) and different protein substrates (43.0; feather meal, residual feather meal, residual biomass or a mixture of feather meal and residual biomass). The protein substrates were added to the medium in agreement with the same amount of protein in the final composition (39.34 g protein per L). The initial pH of the medium was adjusted to 7.0. Erlenmeyer flasks (250 mL) containing 50 mL of medium were inoculated with 500 μL (1%, v/v) of a bacterial suspension and incubated at 30 °C in a rotary shaker (125 rpm) for up to 72 h. Aliquots of the culture medium were collected at pre-determined intervals and clarified by centrifugation (4 °C, 5000×*g* for 20 min) whereas the supernatant with the enzyme was used for monitoring the pH and the enzyme activity. The compounds with antioxidant activity were determined at the end of cultivation after the centrifugation and lyophilization process.

2.2.1. Protease productivity determination

Protease productivity (*P*, U/mL/h) was determined for each growth curve from the maximum values of enzyme activity for each substrate under study. Protease productivity was calculated by Eq. (1).

$$P = (A_{max} - A_0)/t \quad (1)$$

where A_0 is the initial enzyme activity (U/mL), A_{max} is the maximum activity (U/mL) and t is the time required to achieve A_{max} (h).

2.3. Scanning electron microscopy (SEM)

To characterize the degradation of feather meal by *Bacillus* sp. P45, feather meal and feather meal waste samples were freeze-dried and then coated with gold palladium in the Electron Microscopy Centre (CEME-SUL) located at the Federal University