Detection of keratinolytic activity by *Aspergillus* sp. and *Penicillium* sp. species using chicken feather residue as alternative medium

Detecção de atividade queratinolítica por espécies de *Aspergillus* sp. e *Penicillium* sp. usando resíduos de penas de frango como alternativo meio

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ABSTRACT

The use of keratinases is biotechnologically important due the ability of hydrolyze the keratin, main structural constituent of chicken feather. These feathers are by-products of poultry-processing industries, that generate large amounts of waste and cause serious environmental impacts. In this context, the present study aimed to investigate the keratinolytic activity of fungi isolated from Caatinga de Pernambuco (Brazil) using chicken feather residue as substrate. The selection of the fungi Aspergillus sp. and Penicillium sp. for production of keratinase was performed in medium containing different concentrations of chicken feather (0.5%, 1.0% and 1.5%) at 28°C and 35°C. Enzyme activity was detected by visualization of the enzymatic degradation by the halo formation. According with results, after 96h of cultivation the fungi with higher keratinolytic activity was Penicillium sp. UCP 1286 and Penicillium sp. UCP 1288 with enzymatic index of 1.4 and 1.3, respectively, in residue with concentration of 0.5% at 35 °C. The bioprospecting of keratinolytic fungi and the formulation of culture alternative medium using residues contribute with new sources for production of biomolecules for industry.

Keywords: Keratinase, Penicillium sp., Aspergillus sp., residue.

RESUMO

O uso de queratinases é biotecnologicamente importante devido à capacidade de hidrolisar a queratina, principal constituinte estrutural das penas de frango. Essas penas são subprodutos do setor aviário, que geram grande quantidade de resíduos e causam graves impactos ambientais. Nesse contexto, o estudo teve como objetivo investigar a atividade queratinolítica por fungos isolados da Caatinga de Pernambuco utilizando o resíduo de penas de frango como substrato. A seleção dos fungos Aspergillus sp. e Penicillium sp. para a produção de queratinase foi realizada em meio contendo diferentes concentrações de penas de frango (0,5%, 1,0% e 1,5%) a 28 °C e 35 °C. A atividade enzimática foi detectada a partir da visualização da degradação enzimática pela formação do halo. De acordo com os resultados, após 96h de cultivo os fungos com maior atividade queratinolítica foram Penicillium sp. UCP 1286 e Penicillium sp. UCP 1288 com índice enzimático de 1,4 e 1,3, respectivamente, na concentração do resíduo de 0,5% a 35 °C. A bioprospecção de fungos queratinolíticos e a formulação de meio alternative de cultura a partir de resíduos contribui com novas fontes para produção de biomoléculas para as indústrias.

Palavras-chave: Queratinase, Penicillium sp., Aspergillus sp., resíduo de pena de frango.

1 INTRODUCTION

The increase in world production of chicken result in residues such as feathers, viscera, and bone. These residues accumulate in form of garbage after chicken slaughter, undergoing slow degradation, resulting in sulfurous odor compounds. (HU; CHENG; TAO, 2017). On the other hand, the reuse of agroindustrial residues for biotechnological applications has been highlighted due the availability of high added value sources in the production of biomolecules (CALLEGARO, 2018; SILVA et al., 2020).

Feather residue is a rich source of keratins (structural proteins), as well as fat and mineral matter. However, the reuse of this waste generated by the chicken industry remains a challenge in bioconversion processes as they are components of severe degradation (GOUSTEROVA et al., 2005; MACHADO, 2018).

The classification based in biosynthesis and physicochemical characteristics considers keratins into two groups: 'light' and " rigid. The "light" keratins, present in the epidermis, have little cysteine content, being mainly composed of amino acids with small side chains such as glycine, alanine, and serine. "Rigid" keratins have a high concentration of cysteine, with many disulfide bonds and participation of most amino acids, as an example, we can mention bird feathers, with about 10 to 14% cysteine. bonds and participation of most amino acids, as an example, we can mention bird feathers, with about 10 to 14% cysteine. (DAROIT; BRANDELLI, 2014; FERRARO; ANTON; SANTÉ-LHOUTELLIER, 2016; HOU et al., 2017; SINKIEWICZ; STAROSZCZYK;

ŚLIWIŃSKA, 2018). Given this, new technologies have been developed to convert these wastes, thus emerging the interest in studies of methods that enable effective and sustainable treatment (FERRAREZE, 2015).

Bioprocesses using keratinolytic microorganisms have been highlighted as alternative to the processing of these materials, with high added value. The ability of these microorganisms in convert is due the action of keratinases, specific proteolytic enzymes, intracellular or extracellular (PLEISSNER; VENUS, 2016).

Enzyme keratinases (EC 3.4.21/24/99.11) are proteinases that catalyze the hydrolysis of peptide bonds in proteins. Among the biotechnological applications, the microbial keratinase besides of function of hydrolyze keratinous residues can be used in formulation of detergents for textile, pharmaceutical, cosmetics and leather processing, replacing sodium sulfide that is extremely toxic. (DETTMER et al., 2012; JAOUADI et al., 2013). Another possibility of application this enzyme is in animal feed because is considered advantageous over the physical and chemical methods, which use treatment with grinding and cooking for these residues, generating high energy consumption (MÜLLER-HELLWIG et al., 2006).

From a biotechnology standpoint, the prospecting of keratinolytic microorganisms remains one of the main strategies for proposing feather bioconversion processes. Therefore, understanding the functionality, that is, the physiological and metabolic capacities of microorganisms, is fundamental in adaptation of ecological phenomena for technological applications (BOHACZ; KORNIŁŁOWICZ-KOWALSKA, 2019; DAROIT; BRANDELLI, 2014; GUPTA et al., 2013).

Keratinolytic fungi, especially dermatophytes, have been described as being of great medical importance where fragments of their hyphal structures are able to adhere and digest keratin causing poor skin, nail, and hair health (ARAIZA-SANTIBÁÑEZ et al., 2016). On the other hand, the genera of Aspergillus and Penicillium fungi, although considered in some cases opportunistic fungi, are also described as safe organisms and good producers of several enzymes, as well as the production of keratins (BRANDELLI; DAROIT; RIFFEL, 2010; PEREIRA, 2017).

2 MATERIALS AND METHODS

2.1 MICRO-ORGANISMS

Eight samples of fungal cultures of genera Aspergillus sp, coded as UCP (1275, 1276, 1277, 1279, 1357 and 1377) and Penicillium sp., (1286 and 1288) isolated from Caatinga (PE, Brazil), provided by the Culture Collection of the Catholic University of Pernambuco-UCP and registered in World Federation for Culture Collection (WFCC). In order to control the experiments, a culture of fungus with keratinolytic potential was used. Cultures were maintained in Sabouraud Dextrose Agar (SDA) medium of g/L composition: dextrose 40, peptone 10, agar 20, 1000mL distilled water and pH 5.8.

2.2 CHICKEN FEATHER RESIDUE

The chicken feather residue was obtained from poultry company Nutrivil Industria Animal and Feed Trade, located in Caruaru, Pernambuco.

2.3 PREPARATION OF FEATHER MEAL FROM CHICKEN FEATHER RESIDUE

A feather meal of the chicken feather residue with 83.52% keratin protein was purchased commercially. Subsequently, a feather meal was separated on the granulometric sieve (ABERT mm/ µm 300; ABNT / ASTM 50 and TYLER / MESH 48). Then it was stored in plastic containers with thread until use.

2.4 SELECTION OF CULTURES AND DETECTION OF KERATINOLYTIC ACTIVITY

To detect keratinolytic activity a screening was performed using the modified médium (SANGALI; BRANDELLI, 2000). For this, a salt-based agar medium with feather has the following composition in g/L: KH2PO4 2; Mg SO4 1; FeSO4. 7H20 1; MM SO4 1.2; ZMSO4 1.5; agar 15 and feather flour 10, in pH 5.6. After self-washing 121°C for 15 minutes, the culture medium was distributed in Petri dishes.

The inoculum of 50µL fungal cultures from a 107 CFU/mL suspension was transferred to the center of the Petri dishes and then placed in oven at 28 °C for 96 hours. After the microbial growth period, the detection of plaque keratinases was revealed using a 0.1N iodine solution, as well as for comparative purposes the Coomassie Blue dye was tested. All assays were performed in triplicate.

2.5 INFLUENCE OF TEMPERATURE AND RESIDUE CONCENTRATION ON KERATINASE PRODUCTION

To evaluate the effect of temperature and concentration of chicken feather residue, the samples selected through screening. Cultivation was carried out in the medium salt-based agar (g/L: KH2PO4 2; Mg SO4 1; FeSO4. 7H20 1; MM SO4 1.2; ZMSO4 1.5; agar 15) containing feather flour in concentrations of 0.5%, 1.0%, and 1.5g/L varying the temperatures of 28°C and 35°C during 96h.

2.6 ENZYMATIC DETERMINATION

Enzyme determination was expressed as enzymatic index (IE), by the relation of the mean diameter of the degradation halo and the average diameter of the colony (HANKIN; ANAGNOSTAKIS, 1975) according with Equation 1:

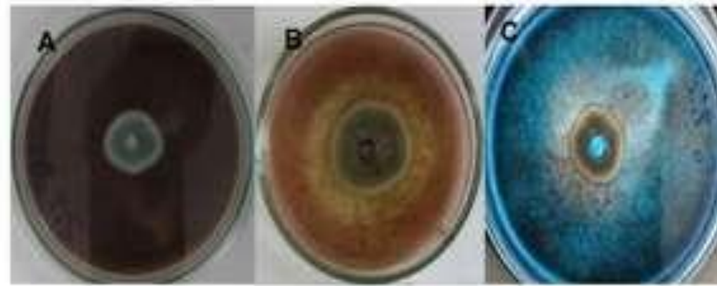
$$IE = \frac{\text{Halo diameter}}{\text{Colony diameter}} \quad \text{Eq.(1)}$$

3 RESULTS AND DISCUSSION

The selection of fungal cultures for keratinolytic activity using feather residue showed that six strains Aspergillus sp. (UCP 1275, 1279, 1357), Penicillium sp. (UCP 1286, 1288) and Keratinolytic fungis Microsporum sp. showed growth in the medium containing feather flour as the sole source of carbon-nitrogen. However, the strains Aspergillus sp. (UCP 1276, 1277 and 1377) did not show growth. The visualization of the keratinolytic activity Penicillium sp. (UCP 1286) in the culture medium obtained by using the different development indicator as show the Figure 1. A clear zone

formation is observed around the colonies, showing the enzymatic degradation in contrast with the iodine and coomassie blue developers.

Figure 1- Detection of keratinolytic activity Penicillium sp. (UCP 1286) (A) control of experiments without degradation halo, (B) development of halo with iodine, (C) development of halo with coomassie blue



After 96h of cultivation, fungi with higher keratinolytic activity were found for Penicillium sp. (UCP 1286 and 1288) cultures with enzymatic index of 1.4 and 1.3, respectively (Figure 2). The variation of the parameters using the Penicillium sp. (UCP 1286) demonstrated that temperature and concentration had effects on the detection of enzymatic activity. The maximum levels of keratinolytic activity were obtained with increasing temperature, but the concentration showed a negative effect oh the activity (Figure 3).

Figure 2 – Keratinolytic activity of Aspergillus sp UCP (1275, 1279, 1357) and Penicillium sp, UCP (1286, 1288) and Microsporum sp. Keratinolytic fungus isolates in 1% chicken feather culture medium for 96 h 28º C

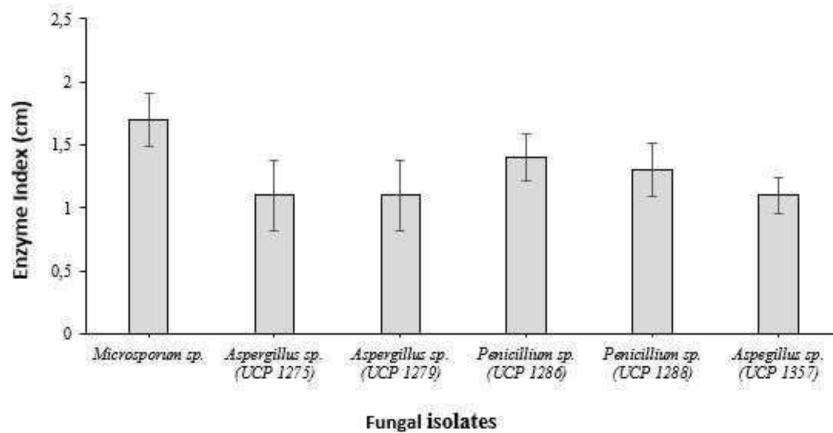
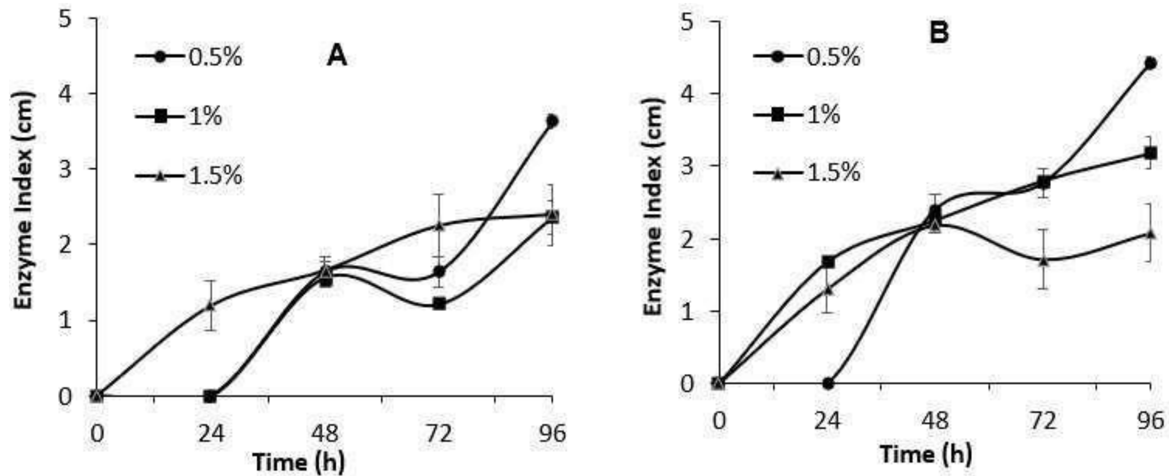


Figure 3 – Keratinolytic activity of Penicillium sp., UCP (1286) fungus at different feather residue concentrations at temératures of 28° C (A) and 35ºC (B) for 96 hours



Screening tests in agar plates for detection of keratinolytic activity in microorganisms have been performed using easy visualization hydrolyzable substrates. The selection of Aspergillus niger fungal strains for keratinase activity was investigated by (MAZOTTO et al., 2013) using detection methods in Petri dishes using coomassie blue reagent. It was verified that the method used for detection allowed the visualization of enzymatic activity.

Due the difficulty in incorporating insoluble substrates such the keratin in medium, most studies use soluble substrates such the gelatin or casein to select keratinolytic microorganisms (KUMAR; KUSHWAHA, 2014). Some bacteria and fungi are able of use keratin as sole source of carbon, sulfur and nitrogen (KIM; LIM; SUH, 2001; KORNIŁŁOWICZ-KOWALSKA; BOHACZ, 2011; SANGALI; BRANDELLI, 2000). The use of keratin-based solid media has been successfully used for the selection of microorganism with potential for keratinase production (SANGALI; BRANDELLI, 2000). Duarte et al., (2009) also performed the selection of keratinolytic Candida strains by feather keratinolytic residues.

The results obtained in this work show that the selected fungus and the feather test half test proved to be efficient for determination of keratinolytic activity. Thus, it was not necessary the extract of keratin from substrate for visualize the degradation halo. On the other hand, it was also observed that the temperature and concentration of the substrate were determining factors in expression of the enzymatic activity of the microorganisms.

4 CONCLUSION

The Penicillium sp. (UCP 1286) was the fungus that showed best potential in production of keratinase in culture alternative medium constituted by chicken feather residue. Thus, this study has

high biotechnological importance by use of fungi with potential of metabolize the chicken feather residue for production of enzyme of industrial interest.

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