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DOI: http://dx.doi.org/10.4322/apa.2015.013

CHARACTERIZATION OF ANTARCTIC KERATINOLYTIC *Arthrobacter* SP

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Abstract: *Keratin is one of most abundant polymers in nature and the main source are feathers. In the poultry industry and in soils, bacteria are responsible for secretion of enzymes that create feather degradation. The diversity of bacteria in Antarctic soils has been studied in the few last years, but the ecological roles of bacteria are poorly understood. In the present work we isolated a bacterium from ornithogenic soil and feather fragments with keratinolytic activity in low temperature (5 °C). Arthrobacter sp. strain PF1 was identified based on morphological and biochemical tests and 16S rRNA sequencing. The bacterium presented optimum growth at 4 and 25 °C, but not at 37 °C. Proteolytic activity was observed at 4 and 25 °C in pH 7 and 10. Our results show a keratinolic bacterium that has feather degradation at low temperature and pH 10, suggesting the production alkaline protease.*

Keywords: Ornithogenic Soil, Feather Degradation, Arthrobacter, Protease

Introduction

Psychrophilic and psycrotolerant bacteria have the ability to grow and colonize environments where the temperature is close to the freezing point of water (Peeters *et al.*, 2011). Temperature in Antarctic is permanently next to zero or below. The ornithogenics soils are rich in organic matter and many studies describe a wide variety of microorganisms when compared with other soils from Antarctica (Aislabie *et al.*, 2009). However, there is no accumulation of the polymers, such as bird feathers. The degradation of polymers, like keratin, is carried out by microorganisms in the soil. In this process it is the secretion of enzymes that degrades keratin.

The aim of this study was to identify new psychrotolerant keratinolytic bacteria showing feather degradation at low temperature. This report describes the identification and keratinase production by *Arthrobacter* sp. strain PF01.

Material and Methods

Isolation and molecular identification

The feather samples were collected of Elephant Island soil (61° 08' S 55° 07' O), South Shetland Islands during XIX Antarctic expedition. The feather fragments were seed in flour agar feather (20 g/L^1, agar 10 g/L^1) and incubated at 4 °C. The single colony were isolated and newly grown on flour agar feather at 4, 25, 37 and 42 °C. Genomic DNA was extracted of isolate PF01 and performed, according to the phenol-chloroform method. The sequence of the 16S rRNA gene (primers were 27f (5´-AAGGAGGTGATCCAGCC-3´) and 1525r (5´-AAGGAGGTGATCCAGCC-3´) was determined by PCR amplification and sequencing. The 1216-bp sequence was submitted to Genbank and the BLAST algorithm was used to search for homologous sequences.

Biochemical tests

For morphological characterization was performed Gram coloration and microscopic observation of bacteria morphology. The biochemical tests used different verification methods for evaluation of metabolic routes: Catalase, Casein Peptone, Soy Peptone, Azida Dextrose, Triple Sugar Iron (Glucose, Lactose, Saccharose and H_2S production), Methyl Red, Voges Proskauer, Indole, Motility, Phenylalanine, Simmons' citrate and Mannitol.

Proteolytic tests

Proteolytic activity was tested by Keratin and Casein hydrolysis. Means containing casein (yeast extract 3 g/L^1, meat peptone 5 g/L^1, skim milk 100 mL/L^1, agar 12 g/L^1) were seeded with colonies and grown during 7 days at 4, 25, 30 and 40 °C. The pH alteration was performed for acid and alkaline means. As positive control were used a *Bacillus cereus* ATCC strains.

Results

The PF1 keratinolytic bacteria isolated from decomposing feathers showed vigorous growth in agar feather meal at 4 and 25 °C, but not at 30 °C. The PF1 was not able grow at 37 °C. The results of taxonomic studies on isolated strain PF1 are summarized in Table 1. The genus determination based in phylogenetic analyses of the 16S rDNA. The sequence of gene showed high similarity with *Arthrobacter gangotriensis* (99 %), and among sequences of isolates of the same species have been identified, 100%. The biochemical test and morphological characteristics are compatible with this genre.

Keratinase activity of PF1 was observed after 1 hour at 4 °C, but more pronounced at 25 °C (Figure 1). After 24 hours PF1 strain increased its proteolytic activity. The effect of pH was determined and enzyme have more activity at pH 10 (Figure 2).

Table 1. Results of morphological and biochemical test of strain PF1.

Morphological characteristics	
Gram stain	Positive
Spore	Nos-sporulating
Cultural characteristics	
Feather meal agar colonies	Yellow color
Physiological characteristics	
Catalase	Positive
Citrate	Negative
Voges Proskauer test	Negative
Motility	Positive
Manitol	Negative
Glicose	Positive
Sacarose	Negative
Lactose	Negative
H_2S	Negative

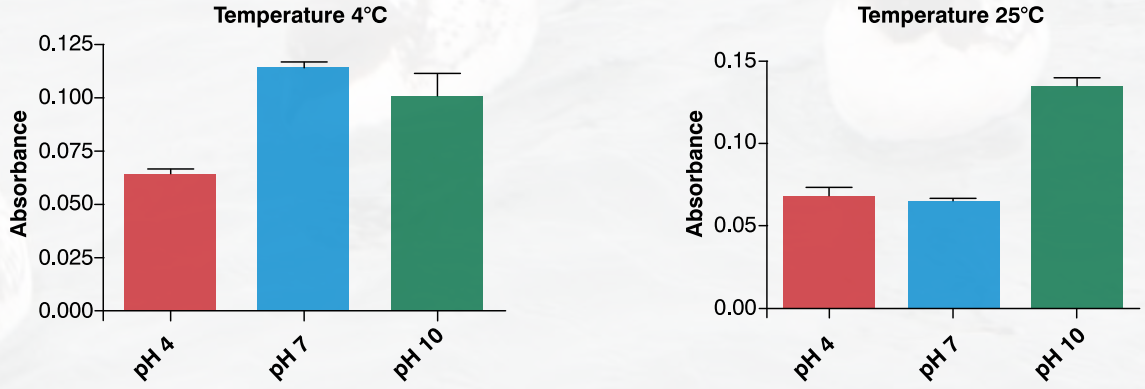

Figure 1. Keratinolitic activity of PF1 after 1 hour at 4°C and 25°C, in different conditions of pH.

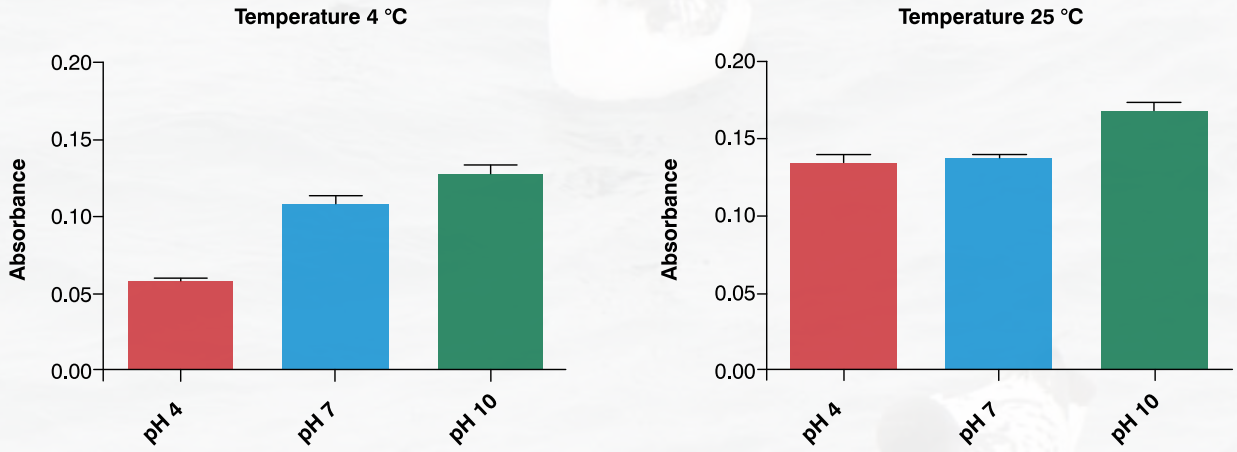

Figure 2. Keratinolitic activity of PF1 after 24 hours at 4 °C and 25 °C, in different conditions of pH.

Discussion and Conclusion

A feather-degrading bacterium was isolated from penguin feather collected on Elephant Island. Based on 16S rDNA sequence PF1 strain belongs to *Arthrobacter*. The similarity with *A. gangotriensis* was 99 %. *Arthrobacter* species have been described in ornithogenic (Aislabie *et al.*, 2009) and although not present, very high proteolytic activity was shown to have the ability to degrade keratin in low temperatures in pH alkaline. The main proteolytic activity of keratinases is normally associated with serine proteinase activity (Lin *et al.*, 1995).

The new strain of *Arthtobacter* sp described here has keratinolytic activity and is effective in feather degradation at low temperature, suggesting its potential use in biotechnological process involving protein hydrolysis.

Acknowledgements

This work is supported by the National Institute of Science and Technology Antarctic Environmental Research (INCT-APA) that receives scientific and financial support from the National Council for Research and Development (CNPq process: n° 574018/2008-5) and Carlos Chagas Research Support Foundation of the State of Rio de Janeiro (FAPERJ n° E-16/170.023/2008). The authors also acknowledge the support of the Brazilian Ministries of Science, Technology and Innovation (MCTI), of Environment (MMA) and Inter-Ministry Commission for Sea Resources (CIRM).

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