BRAZILIAN JOURNAL OF MICROBIOLOGY XXX (2016) XXX–XXX

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Review

Collagenolytic enzymes produced by fungi: a systematic review

Maria Carolina de Albuquerque Wanderley[a], José Manoel Wanderley Duarte Neto[a], José Luiz de Lima Filho[a], Carolina de Albuquerque Lima[b], José António Couto Teixeira[c], Ana Lúcia Figueiredo Porto[d,*]

[a] Federal University of Pernambuco, Laboratory of Immunopathology Keizo Asami (LIKA), Recife, PE, Brazil
[b] University of Pernambuco-UPE, Faculty of Science, Education and Technology of Garanhuns, Garanhuns, PE, Brazil
[c] University of Minho, Centre of Biological Engineering, Braga, Portugal
[d] Federal Rural University of Pernambuco, Department of Morphology and Animal Physiology, Recife, PE, Brazil

ARTICLE INFO

Article history:
Received 29 December 2015
Accepted 15 August 2016
Available online xxx

Associate Editor: Adalberto Pessoa

Keywords:
Collagenase
Fungus
Characterization
Purification
Production

ABSTRACT

Specific proteases capable of degrading native triple helical or denatured collagen have been required for many years and have a large spectrum of applications. There are few complete reports that fully uncover production, characterization and purification of fungi collagenases. In this review, authors searched through four scientific on line data bases using the following keywords (collagenolytic OR collagenase) AND (fungi OR fungus OR fungal) AND (production OR synthesis OR synthesize) AND (characterization). Scientific criteria were adopted in this review to classify found articles by score (from 0 to 10). After exclusion criteria, 21 articles were selected. None obtained the maximum of 10 points defined by the methodology, which indicates a deficiency in studies dealing simultaneously with production, characterization and purification of collagenase by fungi. Among microorganisms studied the non-pathogenic fungi *Penicillium aurantiogriseum* and *Rhizoctonia solani* stood out in volumetric and specific collagenase activity. The only article found that made sequencing of a true collagenase showed 100% homology with several metalloproteinases fungi. A clear gap in literature about collagenase production by fungi was verified, which prevents further development in the area and increases the need for further studies, particularly full characterization of fungal collagenases with high specificity to collagen.

* *Corresponding author at:* Department of Morphology and Animal Physiology, Federal Rural University of Pernambuco, Av. Dom Manoel de Medeiros, s/n, 52171-900 Recife, PE, Brazil.
E-mail: analuporto@yahoo.com.br (A.L. Porto).

http://dx.doi.org/10.1016/j.bjm.2016.08.001

Please cite this article in press as: Wanderley MC, et al. Collagenolytic enzymes produced by fungi: a systematic review. *Braz J Microbiol.* (2016), http://dx.doi.org/10.1016/j.bjm.2016.08.001

Introduction

Collagen is a fibrous protein found in skin, tendons, bones, teeth, blood vessels, intestines and cartilage, corresponding to 30% of the total protein, whose main function is structural.[1,2] There are more than 26 genetically distinct types of collagens, characterized by considerable complexity and diversity in their structure, their splice variants, presence of additional, non-helical domains, their assembly and their function.[3,4] Each collagen molecule is a small, hard stick formed by interlacing in a triple helix of three polypeptide chains called alpha chains (Fig. 1).

Specific proteases capable of degrading native triple helical or denatured collagen have been required for many years.[5] Collagenases have been isolated and characterized from different sources, as digestive tracts of fish and invertebrates including: tadpole tailfin,[6,7] Atlantic cod,[8] land snail (*Achatina fulica*),[9] tropical shrimp (*Penaeus vannamei*),[10,11] catfish (*Parasilurus asotus*),[10,12] mackerel (*Scomber japonicas*)[13]; besides plants (*Zingiber officinale*)[14]; bacteria as: *Bacillus cereus* and *Klebsiella pneumoniae*,[15] *Bacillus pumilus*,[16] *Bacillus licheniformis*[17–19] and fungi, shown in this review.

Proteases, in general, from microbial sources are preferred to the enzymes from plant and animal sources for its biochemical diversity and genetic manipulation possibility.[20,21] Microbial collagenase have been recovered from pathogenic micro-organisms, especially *Clostridium histolyticum*, which is the most widely used commercial source.[22] Other studies reported collagenase producing fungi of genera *Aspergillus*, *Cladosporium*, *Penicillium* and *Alternaria*.[23]

Among microorganisms that produce collagenolytic enzymes, filamentous fungi have great advantages such as high productivity and low production cost, rapid development, and the resulting enzyme may be modified and recovered more easily.[24] Enzyme production occurs extracellularly, which makes it particularly easier to recover afterwards.[25] As fungal proteases are capable of hydrolyzing many other proteins besides collagen, the demand for collagenases from fungi with suitable characteristics, namely high specificity, is a very significant research direction to be taken.[26] Collagenases are capable of hydrolyzing both native and denatured collagen, and are becoming increasingly important commercially.[27]

Collagenases have been used in medical, pharmaceuticals, food, cosmetics and textiles segments and have applications in fur and hide tanning to help ensure the uniform dying of leathers.[28,29] In medical applications, it can be used in burns and ulcers treatment,[30,31] to eliminate scars,[32] for Dupuytren's disease treatment in addition to various types of fibrosis such as liver cirrhosis, to preparing samples for diagnosis,[33] for production of peptides with antioxidant and antimicrobial activities,[34] and play an extremely important role in the transplant surgery success of some specific organs.[32]

Fig. 1 – Collagen molecule: intertwining three alpha chains triple helix.

The rules for vertebrate collagenase classification are very clear, but the same does not apply to microbial enzymes. It is difficult to distinguish between true collagenases and gelatinases or other proteases, which leads to controversy and imprecision in the classification and nomenclature of these enzymes. Microbial collagenases are capable of degrading triple-helical collagen and denatured fragments in various sites and are less specific. Although several proteases can hydrolyze denatured collagen, they cannot be mistaken with true collagenases, able to hydrolyze the native collagen as found in connective tissues.[35,36]

The search for new microbial collagenases has increased over the years and its production currently represents one of the biggest enzyme industries.[37,38] The development of new production methods, including the search for producing micro-organisms, alternative sources of substrates, and better extraction conditions and purification of collagenase, has been of great importance, since it has a wide application spectrum with high biotechnological potential. Besides, the main published review papers concerning microbial collagenolytic enzymes are limited to bacterial source.[22,35,39] In this context, the authors felt the need to better understand the state of the art regarding production, characterization and purification of collagenolytic enzymes by fungi.

Material and methods

The first step on this process, was to make electronic searches in the Scopus (http://www.scopus.com/), ScienceDirect (http://www.sciencedirect.com/), ISI Web of Science (http://apps.isiknowledge.com) and PubMed (http://www.ncbi.nlm.nih.gov/pubmed) databases, using the following keywords: (collagenolytic OR collagenase) AND (fungi OR fungus OR fungal) AND (production OR synthesis OR synthesize) AND (characterization).

This procedure allowed selecting published papers on the production and characterization of collagenolytic enzyme produced by fungi. Papers that did not report on the enzyme production process were excluded. There were no limitations regarding the year and date of publication, due to lack of publications about this issue. No restrictions were made for methodology used, types of analysis and quantification of results. In addition, there were no restriction on type of micro-organism, collagenolytic activity methodology, culture conditions and characterization assays.

Two independent searches were made and the conformity of the selected papers validated, considering the inclusion criteria described. In case of divergence among the papers, all of the criteria were reviewed and discussed. When in the article title only protease production was mentioned, lacking collagen related terms, researchers proceeded to summary evaluation, looking for methodologies for activity determination involving collagen or gelatin as substrate.

Papers selection criteria were defined to evaluate both better conditions for collagenolytic enzyme production by fungus with biotechnological potential applicability and methodological quality in the characterization of the enzyme. Scientific

Please cite this article in press as: Wanderley MC, et al. Collagenolytic enzymes produced by fungi: a systematic review. *Braz J Microbiol.* (2016), http://dx.doi.org/10.1016/j.bjm.2016.08.001