Biotechnology Reports 12 (2016) 40–51

Contents lists available at ScienceDirect

Biotechnology Reports

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

Effect of amino acids on the repression of alkaline protease synthesis in haloalkaliphilic *Nocardiopsis dassonvillei*

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ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form 5 September 2016
Accepted 11 October 2016
Available online 17 October 2016

Keywords:
Alkaline protease
Halo-alkaliphiles
Marine actinobacteria
Nocardiopsis dassonvillei
Enzyme repression

ABSTRACT

A newly isolated salt-tolerant alkaliphilic actinomycete, *Nocardiopsis dassonvillei* strain OK-18 grows on mineral salts medium with glucose as carbon source. It also grows and produces protease with amino acids as sole carbon source. The synthesis of extracellular alkaline protease parallel to growth was repressible by substrate concentrations. The absolute production of the protease was delinked with growth under nutritional stress, as protease production was high, despite poor growth. When amino acids served as the sole source of carbon and nitrogen, the enzyme production was significantly controlled by the number of amino acids. Maximal protease production was achieved with proline, asparagine, tyrosine, alanine, methionine and valine as sole source of carbon and nitrogen in minimal medium. With the increasing number of different amino acids in the presence and absence of glucose, the protease production was synergistically lower as compared to complex medium.

1. Introduction

Studies on the marine actinobacteria in recent years have emerged as one of the major aspects of research [1]. Actinobacteria have gained significance not only due to their taxonomy, diversity and ecological significance, but also on account of their enzymes, unique metabolites and bioactive compounds [1,2].

Proteases constitute one of the most important groups of industrial enzymes and ubiquitously present in all organisms. They have potential applications in different industries that include laundry detergent, food, pharmaceutical, peptide synthesis, leather, meat processing, silk and silver recovery from used X-ray films [3,4]. The catalysis and stability of the microbial proteases under wide range of conditions including high salinity and alkaline pH is one of the major requirement and challenge. In this context, the enzymes from the haloalkaliphilic organisms that can function at low and high concentrations of NaCl over a broad range of pH appear promising [5].

Microbial alkaline proteases are widely reported from *Bacilli* and *Streptomyces* [6,7]. However, protease production and characterization from actinomycetes, particularly *Nocardiopsis* has not achieved similar attention. Most of the work on actinomycetes relate to antibiotics and other bioactive compounds [8].

Proteases production in microorganisms is generally constitutive or partially inducible. However, only limited knowledge exists on the mechanisms which regulate the protease synthesis and its secretion [9–12]. The production of extracellular serine protease [13] in microorganisms is strongly influenced by the environmental parameters and media components, for instance, variations in C/N ratio, presence/absence of metabolizable sugars, such as glucose [14] and rapidly metabolizable nitrogen sources, such as amino acids. Besides, several other factors, such as aeration, inoculum load, medium pH, temperature and incubation time [15,16] and metal ions [17] play important role in protease synthesis.

Secretion of the alkaline proteases from actinomycetes is dependent on the growth rate and availability of the carbon and nitrogen sources in the medium [18]. Various nitrogen sources including amino acids at certain concentrations repress the enzyme production. Repression of the synthesis of biosynthetic enzymes by the end product of their action is an important aspect of the metabolic regulation in microorganisms. [19]. Enzyme repression is a mode of regulation through which the synthesis of an enzyme is prevented by repressor molecules. As a result, end product acts as a feed-back-co repressor in association with intracellular apo-repressor and blocks the function of an operator [20].

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http://dx.doi.org/10.1016/j.btre.2016.10.004

The production of an enzyme demonstrates a relationship with the growth of the organism. Generally, the protease synthesis and secretion are induced by peptides or other proteinaceous substrates. Amino acids repress protease synthesis even at low concentrations [21]. However, production of protease is controlled by numerous complex mechanisms during the transition of the exponential and the stationary phase [22,23]. Various nitrogen sources are known to repress enzyme production in Gram negative bacteria and Actinobacteria [24,25]. However, similar studies on the repression of the enzymes in haloalkaliphilic actinomycete, *Nocardiopsis dassonvillei* have not been earlier reported.

Many studies on the actinomycetes from the temperate regions have been carried out [1]. Till now, terrestrial soils and sediments are the predominant and widely explored sources for the actinomycetes. However, studies on the salt tolerant haloalkaliphilic actinobacteria are quite rare. Haloalkaliphilic actinomycetes and their proteases from the saline habitats of the coastal Gujarat have been studied during the last several years [2,10,11,26–29]. In the present report, we describe the effect of amino acids on the production of alkaline protease in a newly isolated marine actinomycete, *Nocardiopsis dassonvillei* OK-18 from the Okha Port (22.4667°N 69.0833°E), Arabian Sea.

2. Material and methods

2.1. Chemicals

Casein was purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Gelatin, pure amino acids (L form), Minimal Davis Broth (Ingredients g/L: Dipotassium phosphate, 7.0 g; Monopotassium phosphate, 2.0 g; Sodium citrate, 0.5 g; Magnesium sulphate, 0.1 g; Ammonium sulphate,1.0 g) and other media components were purchased from Hi-Media Laboratories (Mumbai, India). All other chemicals used were of highest purity grade.

2.2. Microorganism and culture conditions

The extracellular alkaline protease producing haloalkaliphilic actinomycete was isolated from sea water near Okha Port (22.4667°N 69.0833°E), Gujarat (India). Sea water samples without dilution were subjected to thermal treatment by heating in a water bath at 60–70 °C for 30–60 min to reduce the number of undeniable bacteria. Actinomycetes were isolated at 30 °C using selective media. Further identification was based on the 16S rRNA gene sequencing of the isolate.

2.3. Enzyme repression by various amino acids

The repressive effect of amino acids was studied as described earlier [26], with some modifications in minimal medium with 5% (w/v) NaCl and 0.5% (w/v) glucose as the sole source of carbon without any other nitrogen source at pH 11.0. The amino acids were selected on the basis of charge, side chain and polarity. The stock solutions of the amino acids were filter-sterilized and added to a final concentration of 0–1% (w/v) in the sterilized minimal medium. Fifty millilitres of the production medium in a 250 mL flask was inoculated with 3% of 48–72 h old grown seed culture (optical density of 1.0 at $540_{nm} \sim 10^7$ cells/mL) and incubated at 30 °C under shaking conditions (120 rev/min) for 264 h. The culture aliquots were collected and centrifuged to obtain cell-free supernatant, followed by the measurement of growth. The cell free culture filtrates were used as a crude enzyme preparation and ratio of enzyme production and growth was calculated.

2.4. Enzyme assay

Alkaline protease was estimated by Anson-Hagihara method [30], using casein as substrate. One unit of the alkaline protease activity (U) was described as the enzyme liberating 1 μg of tyrosine per min under the assay conditions. The estimations were based on a tyrosine calibration curve.

2.5. Effect of the increasing number of amino acids on protease production

In order to investigate the influence of the combinations of the amino acids on protease production, OK-18 was grown in minimal media, pH 11.0, supplemented with 5%, (w/v) NaCl and increasing number of amino acids; in the presence and absence of 0.5% (w/v) glucose. The L-amino acids were added, each at the concentration of 1% (w/v), in the combinations; (1) proline, (2) proline, glutamine, (3) proline, glutamine, valine, (4) proline, glutamine, valine, isoleucine, (5) proline, glutamine, valine, isoleucine, glutamic acid, (6) proline, glutamine, valine, isoleucine, glutamic acid, threonine, (7) proline, glutamine, valine, isoleucine, glutamic acid, threonine, tryptophan, (8) proline, glutamine, valine, isoleucine, glutamic acid, threonine, tryptophan, serine, (9) proline, glutamine, valine, isoleucine, glutamic acid, threonine, tryptophan, serine, arginine hydrochloride, (10) proline, glutamine, valine, isoleucine, glutamic acid, threonine, tryptophan, serine, arginine hydrochloride & glycine. The medium was inoculated with 3% inoculums and after 264 h growth at 30 °C; cell mass and protease were monitored.

2.6. Effect of non polar side chain amino acids on protease production

Combinations of non-polar side chain amino acids were included in the medium as described above. The amino acids, each at the concentration of 1% (w/v), were added in combinations of glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine and tryptophan. The medium was inoculated with 3% inoculums and after 264 h growth at 30 °C; cell mass and protease were monitored as described above.

2.7. Effect of uncharged polar side chain amino acids on protease production

Effect of uncharged polar side chain amino acids on protease production in OK-18 was assessed in minimal mediathat contained 5%, (w/v), NaCl at pH 11.0 in the presence and absence of glucose, 0.5% (w/v). The amino acids, each at the concentration of 1% (w/v), were added in combination of serine, threonine, asparagine, glutamine, tyrosine and cysteine. The medium was inoculated at 3% of the inoculums and after 264 h growth at 30 °C; cell mass and protease were monitored.

2.8. Effect of essential and non essential amino acids on protease production

Effect of essential and non essential amino acids on protease production in OK-18 was assessed in minimal media that contained 5%, (w/v), NaCl at pH 11.0 in the presence and absence of glucose, 0.5% (w/v). The amino acids, each at the concentration of 1% (w/v), were added in combinations of histidine, isoleucine, methionine, phenylalanine, threonine, tryptophan and valine. And for non essential amino acids, each at the concentration of 1% (w/v), were added in combination of alanine, arginine, asparagine, aspartic acid, glutamine, glycine, proline, serine and tyrosine. The medium was inoculated at 3% of the inoculums and after 264 h growth at 30 °C; cell mass and protease were monitored.