Biocatalysis and Agricultural Biotechnology 8 (2016) 139–145

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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

Production of clinically efficient uricase enzyme induced from different strains of *Pseudomonas aeruginosa* under submerged fermentations and their kinetic properties

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ARTICLE INFO

Article history:
Received 25 April 2016
Received in revised form
26 August 2016
Accepted 6 September 2016
Available online 9 September 2016

Keywords:
Uricase
Hyperuricemia
Submerged fermentations (SMF)
Polarized Light Microscopy (PLM)
Kinetic studies

ABSTRACT

Uricase is a therapeutic enzyme used to regulate the concentration of accumulated uric acid in gout disease. The two strains of *Pseudomonas aeruginosa Ph3 & Pseudomonas aeruginosa 5Y2* had shown uricase production upon induction using uric acid (UA) as an inducer. At the optimized parameters, the crude preparations of strain *5Y2* showed comparatively higher uricase activity of 17.77 U/ml than strain *Ph3* of 13.42 U/ml. The uricase production by *5Y2* was observed to be proportionally increased with the UA concentration; however, substrate inhibition was observed beyond the concentration of 8.92 mM of UA in the case of strain *Ph3*. The *in vitro* degradation study of urate crystals by Polarized Light Microscopy (PLM) showed the efficiency of both uricase. Kinetic parameters Km and Vmax for *Ph3* uricase were found to be 9 μg/ml and 7.5 U/ml and for *5Y2* uricase those were 7 μg/ml and 7.77 U/ml.

1. Introduction

Uricase or urate oxidase (EC 1.7.3.3) is an oxidoreductase enzyme that acts on end product of purine metabolism *i.e.* uric acid (UA) which and converts it into highly water soluble compound allantoin. The hyperuricemia condition arises due to the excess accumulation of UA into the joints in the form of monosodium urate crystals which is a sign of inflammatory disease called Gout. Although the gene for uricase enzyme is present in human genome, but it got silenced during evolution (Friedman et al., 1985) due to which UA level get increased. This may be due to the antioxidant properties of UA to increase the life span of hominoids (Álvarez-Lario and Macarrón-Vicente, 2010; Watanabe et al., 2002). The treatment of Gout require a) either the breakdown of UA in water soluble form, so that it can be easily excreted out or b) regulation of UA production by drug allantoin, but it has reported to be hypersensitive in some gout patients. Thus the efforts are being made to break down the UA by uricase enzyme (Bomalaski and Clark, 2004). The major uricase producers are bacteria, fungi, plants and animals excluding human beings.

Microbes are the potential producers of uricase. The optimum parameters including pH, temperature, cofactors, required for uricase activity varies with the type of strain. The reported microbes involved for the uricase production are *Pseudomonas spp.* (Abdel-Fattah et al., 2005; Bongaerts et al., 1977), *Bacillus spp.* (Bongaerts et al., 1978), *Micrococcus* spp. (Nanda and Jagadeesh Babu, 2014), *Streptomyces spp.* (Watanabe et al., 1969), *Candida spp.*, *Gliomastix gueg* (El-Shamy and Nehad, 2010), *etc.*

The objectives of present study were to determine the optimum concentration of inducer UA that produce maximum uricase enzymes from *Pseudomonas* strains under submerged fermentations; to study the effects of physical parameters on uricase activity and to determine the kinetic parameters Km & Vmax of the produced uricase enzymes; and to study *in vitro* degradation of the clumps of UA crystals by Polarized light microscopy.

2. Materials and methods

2.1. Materials

Uric acid, glucose, magnesium sulfate, sodium dihydrogen phosphate and disodium hydrogen phosphate were procured from S D Fine chemicals Ltd. Mumbai, India. Beef extract, yeast extract, phenol red, BSA (bovine serum albumin), agar powder, peptone, manganese sulfate, copper chloride, cobalt sulfate were obtained from Himedia Laboratory Ltd. Bombay, India. Boric acid, sodium

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http://dx.doi.org/10.1016/j.bcab.2016.09.005
1878-8181/

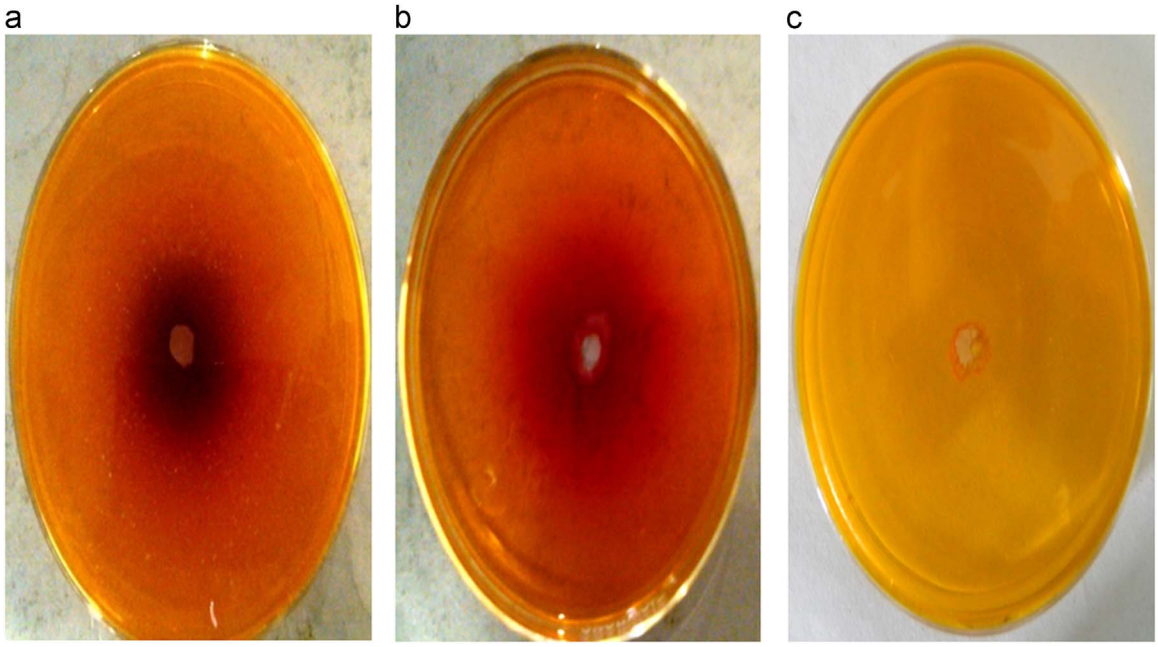

Fig. 1. Plate assay method for extracellular uricase from (A) *P. aeruginosa Ph3,* (B) *P. aeruginosa 5Y2 and* (C) *Control.*

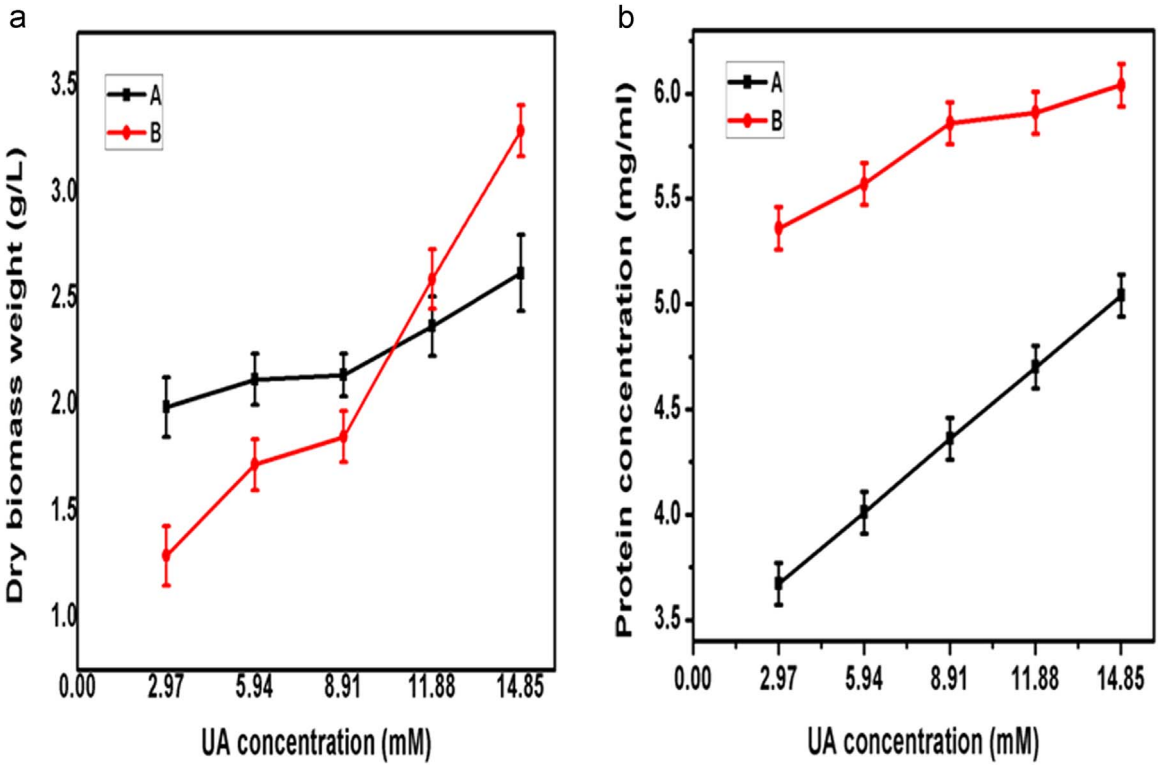

Fig. 2. (a) Quantification of dry weight of biomass of A] *P. aeruginosa Ph3* and B] *P. aeruginosa 5Y2.* (b): Quantification of protein concentration in crude preparations of A] *P. aeruginosa Ph3* and B] *P. aeruginosa 5Y2.*

tetraborate, nickel chloride were purchased from Fischer scientific, Mumbai, India. Sodium chloride, sodium hydroxide, zinc sulfate, iron (III) chloride, copper sulfate pentahydrate, sodium potassium tetrahydrate were procured from Merck Ltd. Mumbai, India. The lyophilized pure cultures of *Pseudomonas aeruginosa Ph3 & 5Y2* (MTCC 7602 and 7199) were procured from MTCC, Chandigarh, India.

2.2. Plate assay method

The capability of microbes to produce extracellular uricase was primarily tested with nutrient agar plate assay method. The nutrient agar plates were prepared with the composition (mg/ml) peptone 5.0, yeast extract 3.0, NaCl 3.5, agar 25.0, phenol red 0.1. To this the inducer UA 8.92 mM was added. Wells of 5 mm diameter were drilled at the center of agar plates. The 200 μL of microbial cultures were added to those made wells. Phenol red dye