Biochemical Engineering Journal 100 (2015) 67–75

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Production of poly-γ-glutamic acid by glutamic acid-independent *Bacillus licheniformis* TISTR 1010 using different feeding strategies

Nuttawut Kongklom [a,b], Hongzhen Luo [c], Zhongping Shi [c], Chiravoot Pechyen [d], Yusuf Chisti [e], Sarote Sirisansaneeyakul [a,b,*]

[a] *Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, 50 Ngam Wong Wan Road, Ladyaow, Chatuchak, Bangkok 10900, Thailand*
[b] *Center for Advanced Studies in Tropical Natural Resources (CASTNAR), National Research University-Kasetsart University, Kasetsart University (NRU-KU), Chatuchak, Bangkok 10900, Thailand*
[c] *The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China*
[d] *Department of Packaging and Materials Technology, Kasetsart University, 50 Ngam Wong Wan Road, Ladyaow, Chatuchak, Bangkok 10900, Thailand*
[e] *School of Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand*

ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form 30 March 2015
Accepted 10 April 2015
Available online 13 April 2015

Keywords:
Poly-γ-glutamic acid (γ-PGA)
Polypeptides
Fed-batch culture
Fermentation
Production kinetics

ABSTRACT

Bacillus licheniformis TISTR 1010 was identified as a glutamic acid-independent producer of poly-γ-glutamic acid (γ-PGA) in a newly modified B medium in a shake flask culture. The fed-batch production of γ-PGA by this strain was optimized through simultaneously and continuously feeding glucose and NH_4Cl with manual and pH-stat based feeding methods in a 7-L stirred fermenter. Using the optimized operation, the dissolved oxygen concentration, the pH, the glucose concentration and NH_4Cl concentration could be stably controlled to extend the γ-PGA production phase and eliminate the hydrolysis of γ-PGA. As a result, the γ-PGA concentration and productivity reached levels of $27.5 \pm 0.2\,g\,L^{-1}$ and $0.286 \pm 0.059\,g\,L^{-1}\,h^{-1}$, respectively. These values of final concentration and productivity were 5-fold and 3-fold of those obtained without the optimization.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Poly-γ-glutamic acid (γ-PGA) is a natural polymer made of D-/L-glutamic acid monomer units linked by amide bonds between the α-amino and the γ-carboxylic acid functional groups. γ-PGA is biodegradable and its degradation products are nontoxic in the environment. As a consequence, γ-PGA is being increasingly used in diverse applications [1–4].

γ-PGA can be produced via fermentation involving species of the bacterial genus *Bacillus*. Some *Bacillus* spp. produce γ-PGA not only intracellularly, but also extracellularly. *Bacillus subtilis* and *Bacillus licheniformis* have been used for producing γ-PGA. The γ-PGA production by *Bacillus* strains can be of two types: either requiring the addition of L-glutamic acid to the fermentation medium, or independent of the externally supplied monomer [3]. Most of the *Bacillus* species assessed for γ-PGA production have required an external supply of L-glutamic acid. These include *Bacillus anthracis* [5], *B. licheniformis* ATCC 9945 [6], *B. subtilis* IFO 3350 [7], *B. subtilis* F-02-01 [8], *B. subtilis* NX-2 [9], *B. subtilis* SAB-26 [10] and *B. subtilis* MJ80 [11]. In these strains, L-glutamic acid added to the culture medium induces the production of intracellular L-/D-glutamic acid monomer units.

In glutamic acid independent strains, all glutamic acid required for making the γ-PGA is generated via the tricarboxylic acid (TCA) cycle (Fig. 1). The glutamic acid units in the cytoplasm are moved to the cell membrane where they are assembled to produce the extracellular γ-PGA [2,12]. The poly-γ-glutamic acid synthetase (Pgs) enzyme complexes PgsBCA embedded in the cell membrane (Fig. 1) are involved in the assembly of the monomer units to γ-PGA. L-Glutamic acid independent producers of γ-PGA include *B. licheniformis* A35 [13] and *B. subtilis* TAM-4 [14]. In these strains, a large amount of γ-PGA is produced from citric acid and an extracellular supply of ammonium via the TCA cycle [15].

* Corresponding author at: Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, 50 Ngam Wong Wan Road, Ladyaow, Chatuchak, Bangkok 10900, Thailand. Tel.: +66 256 25086; fax: +66 2579 4096.
E-mail addresses: nuttawutkong@gmail.com (N. Kongklom), hz.luo@hotmail.com (H. Luo), zpshi@jiangnan.edu.cn (Z. Shi), chiravoot.p@ku.ac.th (C. Pechyen), Y.Chisti@massey.ac.nz (Y. Chisti), sarote.s@ku.ac.th (S. Sirisansaneeyakul).

http://dx.doi.org/10.1016/j.bej.2015.04.007
1369-703X/© 2015 Elsevier B.V. All rights reserved.

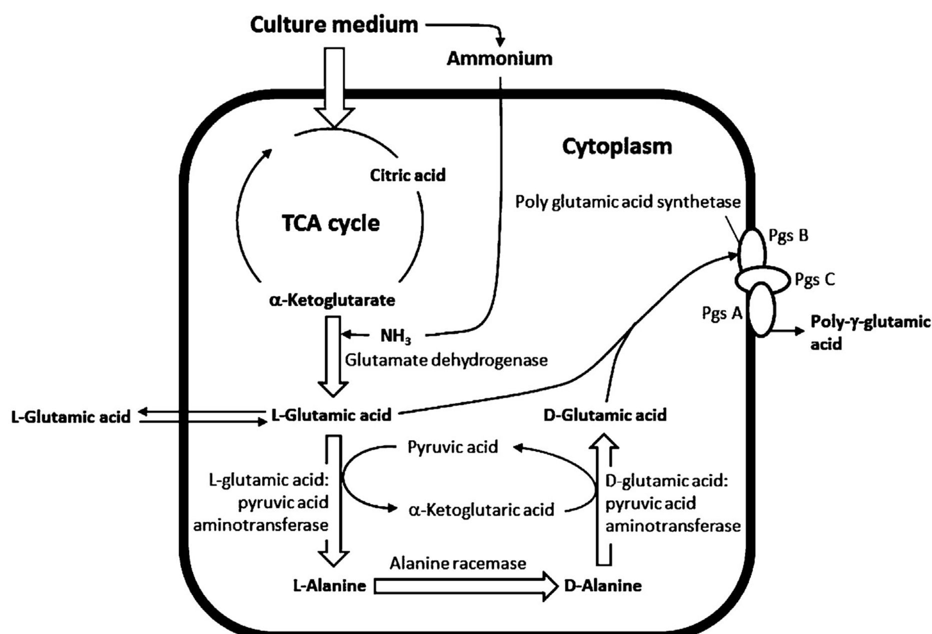

Fig. 1. A proposed pathway for the synthesis of γ-PGA in L-glutamic acid independent *Bacilli* via the tricarboxylic acid (TCA) cycle. Adapted from Shih and Van [1], Buescher and Margaritis [2] and Kunioka [15].

The use of glutamic acid independent strains is preferable for producing γ-PGA as glutamic acid is an expensive substrate compared to inorganic nitrogen sources. In the present work, the bacterium *B. licheniformis* TISTR 1010 is identified as capable of producing γ-PGA without requiring L-glutamic acid in a newly formulated B medium. The γ-PGA production by this bacterium is optimized using a fed-batch operation in a stirred bioreactor involving feeding with a mixed carbon (C) and nitrogen (N) feed. Most studies of γ-PGA production have focused only on batch culture. Here we compare batch and fed-batch operations.

2. Materials and methods

2.1. Screening of γ-PGA-producer strains

Five *Bacillus* strains were grown as pure cultures on NA medium and then inoculated separately into 250-mL flasks containing 25 mL of a modified E medium without L-glutamic acid [16]. The cultures were aerobically incubated at 37 °C in shake flasks (250 rpm) for 48 h. The culture broth was then transferred to 225 mL of a modified E medium without L-glutamic acid in a 500-mL shake flask and incubated under the above conditions for a further 72 h. The fermentation broth was sampled periodically and the cells were removed by centrifugation at 8000-g for 10 min [17]. The cell-free supernatant was hydrolyzed to produce glutamic acid from γ-PGA [18]. The glutamic acid produced was measured via HPLC using a UV–vis detector at 254 nm. A Chirex 3126 (D)-phenicillamine column (150 × 4.6 mm; Phenomenex, USA) was used at 25 °C. The mobile phase was a 85:15 by volume mixture of 2 mM aqueous copper (II) sulfate and methanol at a flow rate of 1 mL min^{-1}.

Based on the concentration of glutamic acid produced by the five screened bacteria, *B. licheniformis* TISTR 1010 (obtained from Thailand Institute of Scientific and Technological Research, TISTR) was determined to be the best producer of γ-PGA [19]. This bacterium was used in all subsequent work.

2.2. Shake flask culture of γ-PGA

B. licheniformis TISTR 1010 was inoculated in 10 mL of Luria-Bertani (LB) medium [20] in a 250-mL flask and aerobically (250 rpm) incubated at 37 ± 0.5 °C for 24 h. The cells were harvested by centrifugation (8000-g, 10 min) and washed aseptically with 0.85% (g/100 mL) of aqueous NaCl. The washed cells were transferred to 100 mL of B medium in a 500 mL shake flask. The B medium comprised of the following (g L^{-1}): glucose 20, citric acid 30, NH_4Cl 7, K_2HPO_4 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, $FeCl_3{\cdot}6H_2O$ 0.04, $CaCl_2{\cdot}2H_2O$ 0.15, $MnSO_4{\cdot}H_2O$ 0.104, NaCl 0.5, 0.3% (w/v, g/100 mL) Tween-80, 1% (v/v) MS vitamin (Murashige and Skoog medium, M3900, Sigma; www.sigmaaldrich.com). The MS vitamin solution contained the following (g L^{-1}): myo-inositol 100, glycine 2, thiamine hydrochloride 0.1, pyridoxine hydrochloride 0.5 and nicotinic acid 0.5. The initial pH was adjusted to 7.4 using sterile NaOH. The culture was incubated at 37 ± 0.5 °C for 72 h on a shaker platform (250 rpm).

2.3. γ-PGA production in 7-L stirred fermenter

B. licheniformis TISTR 1010 was used to inoculate 50 mL of Luria-Bertani (LB) medium in a 250-mL shake flask [20]. Three identical flasks were prepared. The flasks were aerobically (250 rpm) incubated at 37 ± 0.5 °C for 24 h. The content of each flask were used to inoculate 66 mL of fresh LB medium contained in a 500-mL flask. These flasks were incubated as above for 24 h. The cells were aseptically harvested by centrifugation (8000-g, 10 min) and washed with 0.85% NaCl solution. The washed cells from all three flasks were used to inoculate 3.5 L of the earlier specified B medium in a 7 L mechanically stirred fermenter (BIOTECH-7BG, Baoxing Bio-Engineering Equipment Co., Ltd., China). The initial pH was adjusted to 7.4 with NaOH solution. The initial agitation and aeration rates were set at 300 rpm and 1 vvm, respectively. The temperature was controlled at 37 °C throughout the fermentation.

The fermenter was equipped with on-line dissolved oxygen (DO) and pH sensors. The DO and pH values could be automatically recorded. The fermenter featured automatic substrate feeding in response to DO measurement (DO-stat) or pH measurement (pH-stat). These modes of feeding could be automatically activated once the DO or the pH value had exceeded a pre-determined level.

Immediately after inoculation, the DO began to decline because of consumption of oxygen by the cells. To improve oxygen supply, the agitation rate was manually increased in increment of 50 rpm until a maximum value of 550 rpm was attained. If the dissolved

Download English Version:

https://daneshyari.com/en/article/2896

Download Persian Version:

https://daneshyari.com/article/2896

Daneshyari.com