Process Biochemistry 45 (2010) 223–229

Contents lists available at ScienceDirect

Process Biochemistry

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

Localization and production of novel L-asparaginase from *Pectobacterium carotovorum* MTCC 1428

Sanjay Kumar, Veeranki Venkata Dasu *, Kannan Pakshirajan

Biochemical Engineering Laboratory, Department of Biotechnology, Indian Institute of Technology (IIT) Guwahati, Guwahati 781039, Assam, India

ARTICLE INFO

Article history:
Received 1 September 2008
Received in revised form 9 September 2009
Accepted 12 September 2009

Keywords:
Pectobacterium carotovorum
L-Asparaginase
Subcellular fractionation
Localization
Full factorial experimental design
Analysis of variance (ANOVA)

ABSTRACT

Bacterial L-asparaginase has been widely used as therapeutic agent in the treatment of various lymphoblastic leukemia diseases. Studies on localization and production of novel glutaminase-free L-asparaginase were performed using *Pectobacterium carotovorum* MTCC 1428. The localization of L-asparaginase was carried out using cell fractionation techniques. The activity of L-asparaginase was found to be 85 and 77% in the cytoplasm of *P. carotovorum* MTCC 1428 grown on medium containing L-asparagine and combination of L-asparagine and glucose respectively. Among the tested carbon sources, L-asparagine or the combination of L-asparagine and glucose was found to be the most suitable carbon sources to maximize the production of L-asparaginase. The maximum production of L-asparaginase was observed to be 14.56 U/ml (26.92 U/mg of protein) at 4 and 2 g/l of L-asparagine and glucose respectively. Yeast extract, L-asparagine and peptone have shown significant effect on the production of L-asparaginase. *P. carotovorum* MTCC 1428 has assimilated L-asparagine as an essential carbon source for maximizing the production of L-asparaginase.

1. Introduction

L-Asparaginase (L-asparagine amido hydrolase E.C. 3.5.1.1) is an enzyme of high therapeutic value due to its use in certain kinds of cancer therapies, mainly in acute lymphoblastic leukemia (ALL) [1,2]. It is also used in food industry for the production of acrylamide free food [3], model enzyme for the development of new drug delivery system [4] and L-asparagine biosensor for leukemia [5]. Studies on the molecular structure [6], catalysis [7], clinical aspects [2], genetic determinants involved in regulation [8] and stabilization to enhance biological half-life [9] of L-asparaginase have been reported. Many gram-negative bacteria contain two L-asparaginases, a high-affinity periplasmic enzyme and a low-affinity cytoplasmic enzyme. In *Escherichia coli* and many other bacteria, synthesis of cytoplasmic asparaginase I is constitutive, while expression of periplasmic asparaginase II is activated during anaerobiosis. It has been suggested that the latter one probably has a special function in anaerobic fumarate respiration by providing aspartate, which is then converted to fumarate. Further, only the type II enzyme has shown substantial antitumor activity [10]. The antileukemic effect of L-asparaginase is postulated to result from the rapid and complete depletion of the circulating pool of L-asparagine, as most of the cancer cells are dependent on an exogenous source of this amino acid for survival. However, normal cells are able to synthesize L-asparagine and thus are less affected by its rapid depletion due to treatment with this enzyme. The L-asparagine deficiency rapidly impairs the protein synthesis and leads to a delayed inhibition in DNA and RNA synthesis and hence an impairment of cellular function, resulting in cell death [2,11].

In most of the L-asparaginase fermentation processes, the presence of partial glutaminase activity up to 9% of L-asparaginase activity was reported [11]. The various side effects of this drug are mainly due to the presence of partial glutaminase activity [12]. Hence, for successful clinical studies glutaminase-free L-asparaginase is highly desirable. The production of L-asparaginase has been studied in *Serratia marcescens* [13,14], *Erwinia carotovora* [15], *E. coli* [16], *Enterobacter aerogenes* [17], *Pseudomonas aeruginosa* [18], and *Bacillus subtilis* [10] with various carbon and nitrogen sources. The synthesis of L-asparaginase by gram-negative bacteria is regulated by environmental and nutritional factors. The results are contradictory in terms of the effect of glucose [13,16] and oxygen [17] on the production of this enzyme. Factorial designs were applied to study the effects of several factors influencing the responses by varying them simultaneously and carrying out in a limited number of experiments [19–21].

In most of the microorganisms, L-asparaginase accumulates as an intracellular (periplasmic, cytoplasmic and membrane bound) product. The intracellular localization of microbial enzymes has been studied for the production of alkaline phosphatase, deoxy ribonuclease [22], cyclic phosphodiesterase, 5′-nucleotidase, acid

* Corresponding author. Tel.: +91 361 2582212; fax: +91 361 2582249.
E-mail address: veeranki@iitg.ernet.in (V.V. Dasu).

1359-5113/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.procbio.2009.09.011

Table 1
Subcellular distribution of L-asparaginase in various strains.

Strains	Substrate	Cell mass (g/l)	L-Asparaginase activity (U/ml)			Specific activity of L-asparaginase (U/mg)			L-Glutaminase (% cytoplasmic relative activity)
			Cytoplasm	Periplasm	Membrane bound with SDS	Cytoplasm	Periplasm	Membrane bound with SDS	
Serratia marcescens NCIM 2919	Glucose	0.52 ± 0.030	2.83 ± 0.24	1.23 ± 0.22	0.87 ± 0.13	7.25 ± 0.35	3.81 ± 0.59	5.96 ± 0.37	7.68 ± 1.72
	Glucose + L-asparagine	0.50 ± 0.017	12.05 ± 0.76	1.14 ± 0.17	1.48 ± 0.34	31.45 ± 1.34	3.64 ± 0.34	10.20 ± 0.28	7.89 ± 2.10
	L-Asparagine	0.58 ± 0.037	14.07 ± 1.23	0.74 ± 0.36	0.58 ± 0.11	33.73 ± 2.44	2.11 ± 0.40	3.64 ± 0.34	8.90 ± 1.37
Serratia marcescens MTCC 97	Glucose	0.46 ± 0.006	2.87 ± 0.43	0.92 ± 0.27	0.74 ± 0.16	8.36 ± 1.93	2.94 ± 0.77	5.62 ± 0.32	8.12 ± 0.98
	Glucose + L-asparagine	0.42 ± 0.024	10.09 ± 0.96	0.76 ± 0.08	1.19 ± 0.23	30.57 ± 2.22	2.52 ± 0.17	8.35 ± 0.07	6.45 ± 1.32
	L-Asparagine	0.60 ± 0.028	11.81 ± 1.23	1.23 ± 0.15	1.04 ± 0.21	28.13 ± 0.89	2.99 ± 0.83	4.81 ± 0.58	7.70 ± 1.94
Pectobacterium carotovorum MTCC 1428	Glucose	0.53 ± 0.013	1.25 ± 0.23	1.34 ± 0.21	0.41 ± 0.09	3.35 ± 0.49	4.30 ± 0.42	2.66 ± 0.36	0
	Glucose + L-asparagine	0.78 ± 0.014	9.63 ± 0.73	1.32 ± 0.24	0.49 ± 0.06	21.60 ± 1.55	3.61 ± 0.30	2.86 ± 0.65	0
	L-Asparagine	0.76 ± 0.020	10.69 ± 0.62	0.71 ± 0.13	0.38 ± 0.08	22.45 ± 0.65	1.65 ± 0.36	2.29 ± 0.16	0
Wautersia eutropha NRRL B-2804[a]	Glucose	0.36 ± 0.007	2.40 ± 0.22	1.59 ± 0.11	0.88 ± 0.14	7.48 ± 1.14	5.41 ± 0.16	5.97 ± 0.66	2.16 ± 1.37
	Glucose + L-asparagine	0.44 ± 0.021	6.24 ± 0.47	1.24 ± 0.26	1.39 ± 0.11	18.29 ± 0.55	3.63 ± 0.32	8.42 ± 0.10	2.42 ± 1.45
	L-Asparagine	0.55 ± 0.015	5.02 ± 0.32	0.62 ± 0.03	0.48 ± 0.06	15.36 ± 0.93	1.99 ± 0.42	4.30 ± 0.15	1.67 ± 0.93
SK-07 (soil isolate)	Glucose	0.51 ± 0.007	2.05 ± 0.33	1.04 ± 0.16	0.68 ± 0.07	5.38 ± 0.12	3.33 ± 0.32	4.94 ± 0.77	4.11 ± 1.32
	Glucose + L-asparagine	0.46 ± 0.060	6.03 ± 0.79	1.09 ± 0.12	0.75 ± 0.18	16.20 ± 0.99	3.81 ± 0.30	5.67 ± 0.39	3.59 ± 0.45
	L-Asparagine	0.62 ± 0.029	8.46 ± 0.65	0.95 ± 0.10	0.59 ± 0.14	20.79 ± 0.84	2.76 ± 0.37	4.50 ± 0.42	3.78 ± 0.87

Each value = mean value ± S.D.
[a] 16th hour (rest 12th hour).