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Journal of Chemical and Pharmaceutical Research, 2015, 7(6):957-959

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Expression of quinic acid biosynthesis key gene of *Escherichia coli*

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ABSTRACT

Quinic acid is an important compound，it have a wide range of application in many fields. We cloned and expressed the key enzyme of quinic acid metabolism pathway using molecular biology technique, and then increased the number of copies of the key enzymes in E. coli, in order to achieve overexpression of quinic acid to achieve mass production. This method, that improved the target product with enhancing metabolic fluxes, lay the foundation for industrial production for the realization of prokaryotic expression quinic acid.

Key words: quinic acid, metabolic engineering, key gene

INTRODUCTION

Quinic acid (Quinic acid, QA), found in many plants, rarely exist in microorganisms. Currently quinic acid has broad applications in biopharmaceuticals, and then extensively studied. [1,2] With the development of bio-medicine and bio-chemical, quinic acid widely used in many industries such as food, chemical. In addition, there are many other application of quinic acid, such as anti-bacterial, anti-aging and so on [3,4,5,6] . So there is an urgent demand for large-scale production of quinic acid. In this study, we connected the two genes with the prokaryotic expression vector pBV220, after induced in E. coli strain DH5α, achieve the prokaryotic expression, and tested their activity. It laid the foundation for the biosynthesis of quinic acid.

EXPERIMENTAL SECTION

Table 1 Strains and plasmids

Strains and plasmids	Characters	Source and reference
Strains		
*Escherichia coli*31884	Donor of genome	ATCC
Escherichia coli DH5 α	Recipient strain	Tigen公司
Plasmids		
pBV220	AP^r expressing vector	Reference
pBV220-*aroE*	AP^r *aroE*	This work
pBV220-*aroF*	AP^r *aroF*	This work

PCR: We designed two pairs of amplification primers according to Blattner [7] and Lisa [8] reported to amplify *aro*E and *aro*F by PCR from from E.coli 31884 chromosomal DNA and digest with restriction enzyme and sequenced to analysis its correctness.

Gene manipulation techniques and methods, see Ref. [9].
DNA sequence analysis: measured by the sangon biotech.

Gene expression: The plasmid pBV220-*aro*E, pBV220-*aro*F were later transformed into the recipient strain DH5α, cultured overnight and picked strain for PCR validation, then picked the single colony in LB liquid medium (ampicillin) and incubated overnight, expand cultured by 2% to 50mL, cultured at 30℃ until OD600 achieved approximately 0.6, placed in 42 ℃ induced for about 4-5h.

SDS-PAGE: cells were collected by centrifugation at 8000rpm for 10min, boiled 10min and centrifuged at 12000g for 2min, analyzed with 10% separating gel electrophoresis.

Preparation crude enzyme solution of *aro*E and *aro*F: According to the method of cell culture above, after the temperature-induced, cells were collected by centrifugation at 7500rpm for about 15min, sonicated and collected supernatant by centrifugation at 10000g with 4°C for about 30min.

Activity assay of *aro*E and *aro*F: Activity assay of *aro*E and *aro*F were measured according to the literature [10] and [11] respectively.

RESULTS AND DISCUSSION

PCR and sequence analysis of *aroE* and *aroF*
Amplified the *aro*E and *aro*F from genome of *E.coli* 31884, and obtained two fragment with length about 820bp and 1.1kb,Purified the fragments and connected with pBV220, transformed into *E.coli* DH5α, the plasmid were digested with restriction enzyme (Fig. 1) and sequenced the full fragments. the full-length sequence *aro*E consists 819bp, aroF gene consists of 1071bp, screened expressed strains respectively.

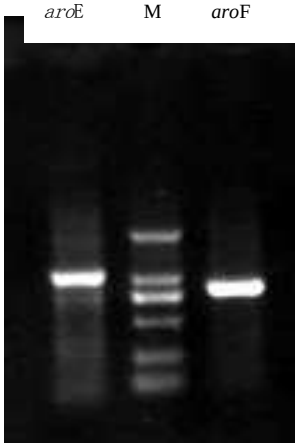

Figure 1 Agarose gel electrophoresis analysis of PCR products M: DL2000

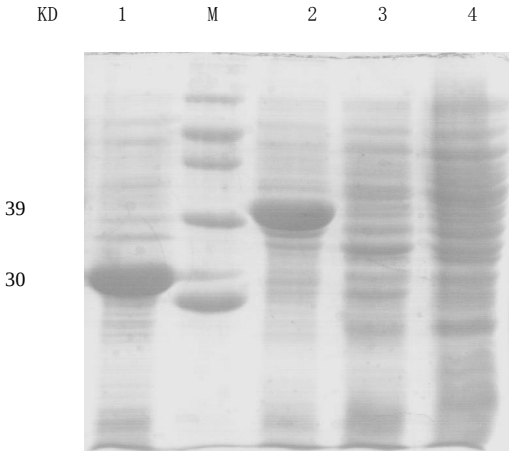

Figure 2 Analysis of *aroE* and *aroF* with SDS-PAGE (10% polyacrylamide gel)
1.DH5α/pbv220-aroF; 2.DH5α/pbv220-aroE; 3.DH5α/pbv220; 4.host DH5α;

Analysis of *aroE* and *aroF* with SDS-PAGE

According to the method above cultured and induced the strains with pBV220-aroE, pBV220-aroF and pBV220 empty plasmid and DH5α host bacteria, SDS-PAGE results shown in Figure2, there were 30kD and 39kD protein band produced in strains with pBV220-aroE and pBV220-aroF respectively.

Activity Assay of *aro*E and *aro*F

Activity assay of *aro*E and *aro*F according to the method above respectively, crude extract of host bacteria which carried pBV220,its enzyme activity ratio is 1.0, host bacteria which carried the pBV220-*aro*E plasmid, its quinic acid dehydrogenase enzyme activity increased by 5.6 times, host bacteria which carried the plasmid pBV220-aroF, its aroF activity improved 9.6 times.

Table 2 Activity of recombinant strain *E.coli* DH5α

Strains /Plasmids	cloning genes	Relative specific activities of enzymes (increased folds)	
		DS	DHQase
E. coli DH5α / pBV220	(Control)	1.0	1.0
E. coli DH5α / PE	*aro*E	1.1	5.6
E. coli DH5α / PF	*aro*F	9.6	1.2

DISCUSSION

Currently, quinic acid still mainly rely on the traditional extraction or chemical synthesis methods, which have the natural disadvantages, such as the yield is relatively low, serious pollution, the synthesis process is relatively complex. enzymatic the later developed method which solves part of the problem, but its high price of substrate and enzyme is not resolved. In this study we combined microbial fermentation with genetic engineering methods to achieve quinic acid biosynthesis, an effective solution to solve the above problems. The use of high density fermentation of *E. coli* can greatly improve the quinic acid synthesis, while the use of genetic engineering to transform quinic acid synthesis pathway, overexpression of the key enzyme in the pathway to achieve a large number of products. In this study, we constructed expression vectors contained *aro*F and *aro*E gene, and the increased DAHP synthase and quinic acid dehydrogenase activity in recipient strain. By increasing the gene copy number to increase the amount of the enzyme, and then increase their activity, which build a foundation for high-yielding engineering strains to produce quinic acid .

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