Original Article

Expression and Characterization of ArgR, An Arginine Regulatory Protein in *Corynebacterium crenatum*[*]

CHEN Xue Lan[1], ZHANG Bin[1], TANG Li[1], JIAO Hai Tao[1], XU Heng Yi[2], XU Feng[2], XU Hong[3], WEI Hua[2], and XIONG Yong Hua[2,#]

1. Key Laboratory of Functional Small Organic Molecule, Ministry of Education, Jiangxi Normal University, Nanchang 330022, Jiangxi, China; 2. State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, Jiangxi, China; 3. Ocean NanoTech, LLC, Springdale, AR72764, USA

Abstract

Objective *Corynebacterium crenatum* MT, a mutant from *C. crenatum* AS 1.542 with a lethal *argR* gene, exhibits high arginine production. To confirm the effect of ArgR on arginine biosynthesis in *C. crenatum*, an intact *argR* gene from wild-type AS 1.542 was introduced into *C. crenatum* MT, resulting in *C. crenatum* MT. sp, and the changes of transcriptional levels of the arginine biosynthetic genes and arginine production were compared between the mutant strain and the recombinant strain.

Methods Quantitative real-time polymerase chain reaction was employed to analyze the changes of the related genes at the transcriptional level, electrophoretic mobility shift assays were used to determine ArgR binding with the *argCJBDF*, *argGH,* and *carAB* promoter regions, and arginine production was determined with an automated amino acid analyzer.

Results Arginine production assays showed a 69.9% reduction in arginine from 9.01±0.22 mg/mL in *C. crenatum* MT to 2.71±0.13 mg/mL (P<0.05) in *C. crenatum* MT. sp. The *argC*, *argB*, *argD*, *argF*, *argJ*, *argG*, and *carA* genes were down-regulated significantly in *C. crenatum* MT. sp compared with those in its parental *C. crenatum* MT strain. The electrophoretic mobility shift assays showed that the promoter regions were directly bound to the ArgR protein.

Conclusion The arginine biosynthetic genes in *C. crenatum* are clearly controlled by the negative regulator ArgR, and intact ArgR in *C. crenatum* MT results in a significant descrease in arginine production.

Key words: *Corynebacterium crenatum*; ArgR protein; Arginine biosynthetic genes; Real-time PCR; Electrophoretic mobility shift assay

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INTRODUCTION

L-arginine (L-Arg), a semi-essential amino acid, has recently attracted considerable attention because of its ability to facilitate the synthesis of various immune active materials for preventing the growth of cancer cells[1], promoting urine circulation, and reducing the ammonia content in blood[2]. As a nitric oxide precursor, L-Arg also has a function in relaxing and dilating blood vessels[3].

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[#]Correspondence should be address to XIONG Yong Hua, Professor. Tel: 86-791-88182405, Fax: 86-791-88333708, E-mail: yhxiongchen@163.com

Biographical note of the first author: CHEN Xue Lan, female, born in 1970, PhD, associate professor, majoring in biochemistry and molecualr biology.

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Therefore, L-Arg is widely used as an additive in the food and pharmaceutical industries.

Corynebacterium crenatum AS 1.542 is a Gram-positive bacterium isolated from soil[4]. Its mutants are widely exploited in the large-scale production of many amino acids, including L-glutamic acid, L-lysine, L-valine, and so on. In our previous work, we successfully obtained a high-yield strain (*C. crenatum* MT) via mutagenesis screening using nitrosoguanidine. This strain was found to possess a greatly enhanced L-Arg-producing ability compared with the wild-type AS 1.542 strain. The L-Arg yield increased from 0 mg/mL to 9.01±0.22 mg/mL. A point mutation was found in the regulator *argR* gene in *C. crenatum* MT: a C-to-T exchange at position 109 in *argR*, leading to an amino acid replacement of Gln-37 (CAG) by a terminator codon (TAG) and thus a truncated ArgR protein.

ArgR is considered as a regulator in the L-Arg biosynthetic pathway (Figure 1)[4-5]. For example, ArgR cooperating with L-Arg in *Escherichia coli* inhibits the transcription of genes participating in L-Arg biosynthesis at different levels and activates the L-Arg degradation pathway[6]. It also has an important function in regulating the arginine-inducible uptake of L-Arg and ornithine metabolism in *Pseudomonas aeruginosa* under aerobic conditions. ArgR activates the *aotJQMOPargR* operon encoding a protein complex for L-Arg and ornithine uptake. ArgR is also a repressor of three operons (*argF*, *carAB*, and *argG*) in L-Arg biosynthesis and two operons (*gltBD* and *gdhA*) in glutamate biosynthesis[7-8]. The mRNA amounts of *argCJBDFR* and *argGH* are reportedly 10 to 44 times higher in a *Streptomyces clavuligerus* *argR*-disrupted mutant than in the wild type, confirming the existence of an ArgR-mediated gene regulation in L-Arg biosynthesis[9]. These results indicate that the ArgR protein is a vital and multifunctional regulator in L-Arg metabolism.

Xu et al. reported that overepression of the arginine biosynthetic *argC-H* cluster from *C. crenatum* with a lethal *argR* gene could improve the production of L-Arg. However, the characterization of *argR* had not been elaborated in detail[10]. This study aims to elucidate further the function of ArgR in L-Arg biosynthesis in *C. crenatum* and to provide basis for the future genetic engineering of L-Arg-producing bacterial strains. Our study focuses on characterizing the *C. crenatum* ArgR protein and its role in the transcriptional regulation of L-Arg biosynthetic gene expression in *C. crenatum* by reconstructing the wild-type *argR* gene in *C. crenatum* MT.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Media

The bacterial strains and plasmids used in this work were described in Table 1. LB medium was used for cultivation of *E. coli* DH5α *and* BL21 (DE3) strains. Seed medium (per liter) for cultivation of *C. crenatum* consisted of 30 g glucose, 20 g cornsteep liquor, 20 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, and 1.5 g urea. Fermentation medium (per liter) for L-Arg production was composed of 120 g glucose, 40 g cornsteep liquor, 20 g $(NH_4)_2SO_4$, 1.5 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.02 g $FeSO_4{\cdot}7H_2O$, 0.05 g $MnSO_4{\cdot}2H_2O$, 8×10^{-5} g biotin, 5×10^{-4} g histidine, and 30 g $CaCO_3$.

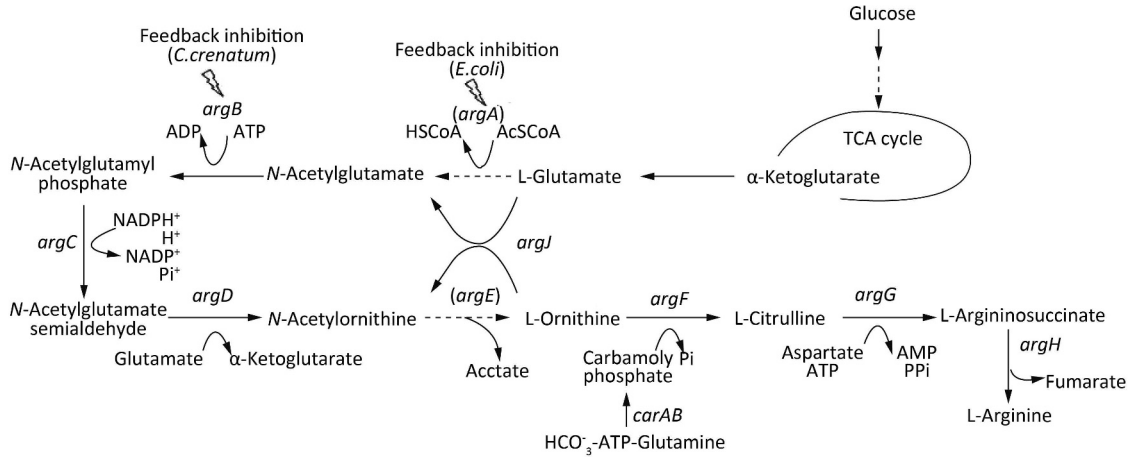

Figure 1. Illustration of the arginine biosynthetic pathway. The difference between the linear pathway and the cycle pathway is that the *argE* gene encodes acetylornithine deacetylase in the linear pathway, whereas the *argJ* gene encodes acetylornithine transferase in the circular pathway.