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Improvement of L-Arginine Production by Overexpression of a Bifunctional Ornithine Acetyltransferase in *Corynebacterium crenatum*

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Abstract Ornithine acetyltransferase (EC 2.3.1.35; OATase) gene (*arg*J) from the L-arginine-producing mutant *Corynebacterium crenatum* SYPA5-5 was cloned, sequenced, and expressed in *Escherichia coli* BL21 (DE3). Analysis of the *arg*J sequence revealed that the *arg*J coded a polypeptide of 388 amino acids with a calculated molecular weight of 39.7 kDa. In this study, the function of the OATase (*arg*J) of *C. crenatum* SYPA5-5 has been identified as a conserved ATML sequence for the autolysis of the protein to α- and β-subunits. When the *arg*J regions corresponding to the α- and β-subunits were cloned and expressed separately in *E. coli* BL21, OATase activities were abolished. At the same time, a functional study revealed that OATase from *C. crenatum* SYPA5-5 was a bifunctional enzyme with the functions of acetylglutamate synthase (EC 2.3.1.1, NAGS) and acetylornithine deacetylase (EC 3.5.1.16, AOase) activities. In order to investigate the effects of the overexpression of the *arg*J gene on L-arginine production, the *arg*J gene was inserted into pJC*tac* to yield the recombinant shuttle plasmid pJC*tac-arg*J and then transformed into *C. crenatum* SYPA5-5. The results showed that the engineered strains could not only express more OATase (90.9%) but also increase the production of L-arginine significantly (16.8%).

Keywords L-Arginine · *Corynebacterium crenatum* · *arg*J · Ornithine acetyltransferases, · Overexpression

Introduction

L-Arginine, a substantial intermediate metabolite in the ornithine cycle, is a kind of semi-dispensable amino acid and has a significant effect in maintaining a normal physiologic

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function in an organism. Therefore, L-arginine has numerous applications in food, pharmaceutical, and cosmetic industries [1]. *Corynebacterium crenatum* SYPA5-5, which is a new L-arginine hyper-producing strain, was isolated from a soil sample and mutated by UV. In our previous studies, the strain, which is auxotrophic for L-histidine, could produce L-arginine at a level of up to 30.6 gL^{-1} in fermentation broth [2].

There are three routes for the biosynthesis of L-arginine from L-glutamate, as shown in Fig. 1 [1, 3, 4]. Route I is a linear pathway existing in Enterobacteriaceae [5] and *Myxococcus xanthus* [6]. In this route, ornithine is directly produced from *N*-acetylornithine by a deacylase, acetylornithine deacetylase (AOase; *arg*E-encoded). Many more organisms, including yeasts, algae, plants, and many bacteria—such as *Bacillus stearothermophilus* [7], *Saccharomyces cerevisiae* [8], *Thermotoga maritime* [9], *Pseudomonas aeruginosa* [10], and *Corynebacterium glutamicum* [11]—have a more evolved cyclic variant of this route in which the acetyl group is recycled by its transfer from acetylornithine to glutamate (route II). In route II, the formation of ornithine is catalyzed by ornithine acetyltransferase (OATase; *arg*J-encoded) transferring the acetyl group of *N*-acetylornithine to glutamate [12]. Route III is a new scarce pathway as in *Xanthomonas campestris* [13] where *N*-acetylornithine was converted into *N*-acetylcitrulline by acetylornithine carbamoyltransferase (AOTCase; *arg*F′-encoded) and citrulline was then produced from *N*-acetylcitrulline by *arg*E.

In different organisms, OATase has monofunctional and bifunctional versions. In a few organisms such as *Streptomyces clavuligerus* [4], OATase is only responsible for the deacetylation of *N*-acetylornithine as AOase (*arg*E-encoded in *Escherichia coli*), whereas in several organisms such as *Neisseria gonorrhoeae* [14, 15] and *Thermus thermophilus* [16], OATase could also synthesize acetylglutamate using acetyl-CoA or acetylornithine as the acetyl donor, which is similar to the activity of *N*-acetylglutamate synthase (NAGS; *arg*A-encoded in *E. coli*). Ornithine acetyltransferase gene (*arg*J) is one of the genes in the

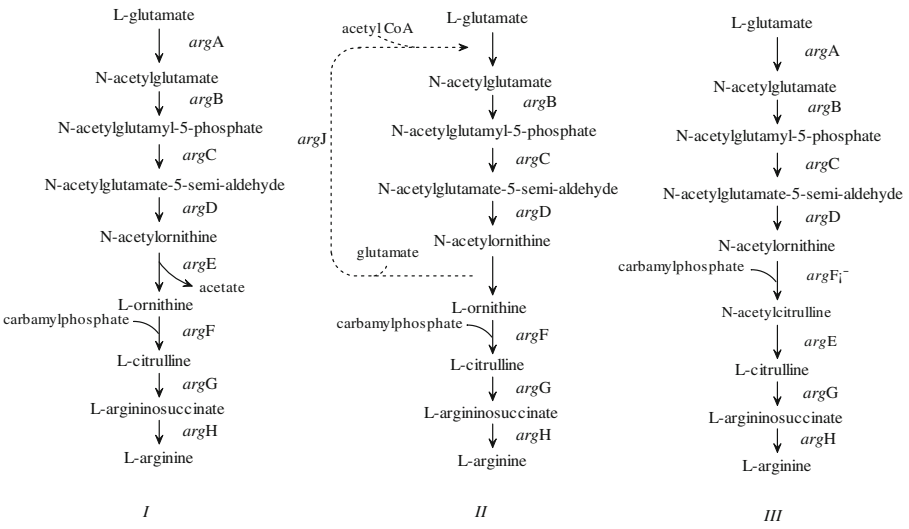

Fig. 1 Biosynthetic pathway of L-arginine. *I* Linear pathway as in *Enterobacteriaceae* strain. *II* Recycling pathway as in *C. glutamicum* and *S. cerevisiae*. *III* New pathway as in *X. campestris*. *argA* acetylglutamate synthase, *arg*J ornithine acetyltransferase, *argB* acetylglutamate kinase, *argC* acetylglutamate semialdehyde dehydrogenase, *argD* acetylornithine transaminase, *argE* acetylornithine deacetylase, *argF* ornithine transcarbamylase, *argF′* acetylornithine carbamoyltransferase, *argG* argininosuccinate synthase, *argH* arginosuccinase

*arg*CJBDFRGH gene cluster that is involved in the synthesis of L-arginine in *Corynebacterium* strains [17]. However, in *C. glutamicum*, the *arg*J-encoded product had been considered as a monofunctional enzyme and lacks NAGS activity [11]. Recently, the overexpression of the *arg*J, exhibiting NAGS activity, was able to complement the *C. glutamicum* arginine auxotrophic *arg*J strain and showed increased NAGS activity [18]. Whether or not OATase is a bifunctional enzyme involved in the acetyl cycle, it plays a vital role in the L-arginine biosynthetic pathway [19].

In this study, *C. crenatum* SYPA5-5 *arg*J gene-encoded OATase was expressed in *E. coli*, and its sequence, primary structure, and function were investigated. Furthermore, we constructed a recombinant shuttle plasmid pJC*tac*-*arg*J for the overexpression of the *arg*J gene and studied its effects on OATase activity and L-arginine production. Our results demonstrated that *arg*J gene-encoded OATase was a bifunctional key enzyme for L-arginine biosynthesis in *C. crenatum*.

Materials and Methods

Strains and Plasmids

The bacterial strains and plasmids used in this work are listed in Table 1.

Plasmids were constructed in *E. coli* JM109 from PCR-generated fragments (*Ex*Taq DNA Polymerase, TaKaRa) using *C. crenatum* SYPA5-5 DNA as a template prepared according to the methods described in [20]. *E. coli* was transformed by the calcium chloride method and *C. crenatum* via electroporation [2, 21]. All recombinant plasmids were analyzed by double digestion analysis and PCR with appropriate primers, respectively.

In order to construct pET-28a-*arg*J and pJC*tac*-*arg*J, the *arg*J gene was amplified by PCR using the upstream primer P*arg*J F (5′-ACGGGATCCATGGCAGAAAAAGGCATTAC-3′; the introduction of a *Bam*HI restriction site was underlined) and the downstream primer P*arg*J R (5′-ACCGGTCGACTTAAGAGCTGTACGCGGAG-3′; the introduction of a *Sal*I restriction site was underlined). The PCR fragment was cloned into the pMD19-T vector, the obtained plasmid pMD19-T-*arg*J was confirmed by digestion with *Bam*HI and *Sal*I, and the *Bam*HI/*Sal*I fragment of *arg*J from pMD19-T-*arg*J was then inserted into the corresponding sites of pET-28a and pJC*tac*, resulting in plasmids pET-28a-*arg*J and pJC*tac*-*arg*J.

To construct pET-28a-*arg*Jα and pET-28a-*arg*Jβ, the *arg*Jα and *arg*Jβ fragments were amplified by PCR using the upstream primers P*arg*JF and P*arg*JBF (5′-ACCGGGATCCAC-CATGCTGGTCTGCTTG-3′; the introduction of a *Bam*HI restriction site is underlined), respectively, and the respective reverse primers P*arg*JAR (5′-ACCGGTCGACTTAGGCAA-GAGACGGCGCCAT-3′; the introduction of a *Sal*I restriction site is underlined) and P*arg*JR. The PCR fragments were cloned into the pMD19-T vector, resulting in plasmids pMD19-T-*arg*Jα and pMD19-T-*arg*Jβ. Then, the isolated fragments were ligated into *Bam*HI/*Sal*I-treated pET-28a, resulting in plasmids pET-28a-*arg*Jα and pET-28a-*arg*Jβ.

Growth Conditions

Luria–Bertani (LB) medium was used as the standard medium for *E. coli*, while LB with 0.5% glucose was used as the medium for *Corynebacterium*. As *C. crenatum* competent cell medium, LB including 3% glycine and 0.1%Tween was used [21, 22]. Slant medium, seed medium, and fermentation medium in a shake flask and in a bioreactor (BIOTECH-5BG, Baoxing Co., China) of *C. crenatum* was described by Xu [2, 23]. When appropriate,

Table 1 Strains and plasmids used in this work

Strain and plasmid	Characteristics	Source
Strains		
Escherichia coli JM109		Takara
Escherichia coli BL21(DE3)		Novagen
Corynebacterium glutamicum ATCC 13032		ATCC
Corynebacterium crenatum SYPA5-5		This study
Plasmids		
pMD19-T	Clone vector, 2.7 kb, Amp^R, *lac*Z	TaKaRa
pET-28a	*E.coli* expression vector, Km^R, T7 promoter	Novagen
pJC*tac*	*C. glutamicum–E. coli shuttle vector,* Km^R, *tac* promoter	[23]
T-*arg*J	A derivative of pMD19-T, Amp^R, harboring the *arg*J gene	This study
T-*arg*Jα	A derivative of pMD19-T, Amp^R, harboring the *arg*Jα gene	This study
T-*arg*Jβ	A derivative of pMD19-T, Amp^R, harboring the *arg*Jβ gene	This study
pET-28a-*arg*J	A derivative of pET-28a, Km^R, harboring the *arg*J gene	This study
pET-28a-*arg*Jα	A derivative of pET-28a, Km^R, harboring the *arg*Jα gene	This study
pET-28a-*arg*Jβ	A derivative of pET-28a, Km^R, harboring the *arg*Jβ gene	This study
pJC*tac-arg*J	A derivative of pJC*tac*, Km^R, harboring the *arg*J gene	This study

the recombinant strains were cultured with ampicillin (100 μg mL^{-1}) or kanamycin (50 μg mL^{-1}).

For L-arginine production experiments, *C. crenatum* strains were activated in slant medium. After 24 h, the seed was inoculated from agar slants and cultured at 30°C for about 15 h in shake flasks. The shake flask culture was then transferred into a 5-L bioreactor; the batch fermentation was performed at 30°C for 96 h. The aeration rate was controlled at 1 vvm, and the agitation rate was 600 rpm for all the experiments. The glucose fed-batch was tested on the flow rate of glucose at 0.9 gL^{-1} h^{-1} from 24 to 80 h. Strain harboring the recombinate plasmid pJC*tac-arg*J was cultivated with kanamycin (30 μg mL^{-1}) before being transferred into the bioreactor.

Recombinant Protein Purification

Recombinant *E. coli* BL21 cells carrying the His-Tag *arg*J, *arg*Jα, and *arg*Jβ were cultivated to the log phase in LB, incubated at 4°C for 30 min, and induced by IPTG (0.5 mmol L^{-1}) at 16°C for 10 h. Cells were harvested by centrifugation (6,000×*g*, 4°C, 10 min), washed twice in PBS (50 mM, pH 7.4), and then disrupted by sonication in PBS and centrifuged (18,000×*g*, 4°C, 15 min). The supernatants obtained were purified by affinity chromatography on a Ni/nitriloacetic acid resin (Qiagen).

Molecular masses of purified recombinant proteins were then estimated by SDS-PAGE using standard protein markers (MBI Fermentas). Protein concentration was determined using Bradford quantification kit (Sangon).

Enzymatic Activity Assay

OATase activity was determined by a method modified from the determination of the ornithine transition from *N*-acetylornithine [14]. Assays were performed in mixtures (500 μL) containing 50 μmol Tris–HCl (pH 7.5), 3 μmol L-glutamic acid (adjusted with NaOH to pH 7.5), and 3 μmol *N*-acetylornithine. After adding the enzyme, the reaction was initiated by the addition of 0.2 mg pre-warmed sodium glutamate. After incubation at 30 °C for 10 min, the reaction was stopped by adding 500 μL of ninhydrin reagent, which was prepared immediately before use by mixing two volumes of 1.5% solution of ninhydrin in ethylene glycol monomethyl ether with one volume of 1.2 M citric acid. The reaction mixture was placed in boiling water for 10 min and then cooled in water, adding 500 μL of 4.2 M NaOH, mixed in an alternator, and the contents were then determined by a Microplate reader at 450 nm. One enzyme unit was defined as the amount of enzyme producing 1 μmol of ornithine per minute. Specific activity of purified OATase was defined as enzyme units included in 1 mg protein (1 U/mg protein), and specific activity of crude OATase was defined as enzyme units included in 1 g cell (1 U/g cell).

NAGS activity was analyzed by LC-MS. The sample was prepared by reaction in mixtures described in OATase activity determination after incubation at 30 °C for 30 min; the components were ready to be analyzed by LC-MS. The LC-MS experiments were carried out on Waters Platform ZMD 4000 equipped with an electrospray ionization source. A chromatographic method was developed using a Hillic column (2.1 × 150 mm). The column oven was maintained at 25 °C. The following gradient elution with acetonitrile as "A" and ammonium acetate as "B" was used at a flow rate of 0.3 mL min^{-1}: 0–10 min, 80% A and 20% B; 10–20 min, 20% A and 80% B. Electrospray was performed by setting the needle voltage at 4.0 kV. The capillary temperature was held at 250 °C, with potential of 30 V.

Assays of Cell Concentration, Glucose, and L-Arginine

Cell concentration was firstly monitored at 562 nm and the dry cell weight (DCW) was determined by a pre-calibrated relationship (1 OD=0.375 gL^{-1} DCW). Glucose concentration was measured using an SBA biosensor analyzer. L-Arginine concentration was measured by an Agilent 1100 HPLC under the following conditions: column Hypersil ODS-C^{18}, 4× 125 mm; temperature, 40 °C; flow rate, 1.0 mL min^{-1}; detection fluorescence detector, Ex 340 nm Em 450 nm; eluent A, 20 mmol L^{-1} Na-acetate; eluent B, 20 mmol L^{-1} of Na-acetate/methanol/acetonitrile, 1:2:2 (*v*/*v*). All of the measurements, particularly the most important state variables—such as the concentrations of cells, L-arginine, and glucose—were measured in three parallel, and the average were collected and used for calculating the "secondary rate variables," including growth rate, L-arginine production rate, and the yield of L-arginine, for all of the jar fermentation experiments conducted. $Y_{P/X}$ is defined as the quantity of L-arginine production (grams)/DCW (grams) and $Y_{P/S}$ is defined as the quantity of L-arginine production (grams)/glucose consumption (grams).

Nucleotide Sequence Accession Number

The nucleotide and amino acid sequences for the *C. crenatum* SYPA5-5 *arg*J gene have been submitted to GenBank with accession no. FJ827483.

Results

Cloning and Sequence Analysis of the *arg*J Gene from *C. crenatum* SYPA5-5

The *arg*J gene from *C. crenatum* SYPA5-5 was amplified by PCR and subcloned into the vector pMD19-T. Sequence analysis of the *arg*J gene revealed that only one ORF existed, which used ATG as the initiation codon and coded a peptide of 388 amino acids with a calculated molecular weight of 39.7 kDa. The *arg*J gene from *C. crenatum* SYPA5-5 was 98.89% homologous to the *arg*J of *C. glutamicum* ATCC13032 which can accumulation little L-arginine. There were only 13 base differences between the two strains, which resulted in three amino acid changes.

The entire 1.1-kb *arg*J gene was subcloned into pET-28a for expression. The subsequent analysis by SDS-PAGE revealed that when induced by IPTG at 30 °C for 4 h, OATase existed as either an intact molecule (Fig. 2a), whose molecular mass is close to the value deduced from its amino acid sequences (39.7 kDa), or two bands, supposed to be subunits α and β of OATase. When induced at 16 °C for 10 h, OATase existed solely as two bands (Fig. 2a). SDS-PAGE analysis of the purified OATase appeared as two clearly distinct soluble bands (Fig. 2b).

Alignment of the deduced amino acid sequence of OATase from different organisms indicated that the OATases from *S. clavuligerus*, *T. thermophilus*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, and *C. glutamicum* have highly conserved cleavage unit sequences made up of xxxATMLxxx amino acids [7]. In nature, the OATase of *C. crenatum* has the ATML sequence for autolysis of the protein to the two individual subunits. The cleavage site is between the alanine residue and the threonine residue within the conserved ATML sequence, resulting in the OATase of *C. crenatum* being divided into two subunits, i.e., α-subunit (182 AA) and β-subunit (206 AA), which would self-assemble to an active OATase (ExPASy: P62058).

Function Investigation of OATase

L-Glutamic acid and *N*-acetylornithine were used as substrates to assay the catalysis activity of OATase, respectively. The LC-MS results revealed that both acetylglutamate and ornithine were formed during the enzymatic reaction, suggesting that OATase from *C. crenatum* SYPA5-5 was a bifunctional enzyme with both AOase activity (synthesis acetylglutamate) and NAGS

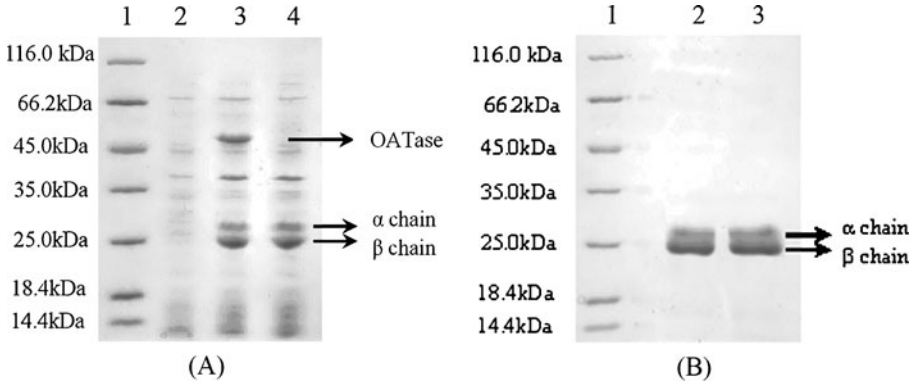

Fig. 2 SDS-PAGE analysis of proteins in whole cells and recombination OATase. **a** Protein Marker (*1*); induced *E. coli* BL21 (pET-28a) by IPTG (*2*); induced BL21 (pET-28a-*arg*J) by IPTG at 30°C for 4 h (*3*); induced BL21 (pET-28a-*arg*J) by IPTG at 16°C for 10 h (*4*). **b** Protein marker (*1*); purified recombinate OATase (*2*, *3*)

activity (synthesis ornithine). Peaks A and B in Fig. 3a are the isomeride of *N*-acetylglutamate. The MS spectrum of peaks A and B displays the same signals of the molecular ions $[M + H]^+$ at *m*/*z* 189. The peak at *m*/*z* 211 is due to $[M + Na]^+$. Because of the low response of peak B in Fig. 3a, the signals obtained in the MS analysis were not intense. Therefore, the peaks at *m*/*z* 224 and 252 correspond to impurities contained in the mobile phase. Figure 3b provided previously was inappropriate and was modified in the revised version.

From the results above, it is suggested that the OATase in the arginine circular pathway from *C. crenatum* catalyzes the two reactions synergistically. A question arose whether OATase was able to show *arg*A or *arg*E activity separately in circular pathway, that is to say, ornithine would be produced from *N*-acetylornithine when L-glutamate was not added. For this question, we used *N*-acetylornithine as the only substrate and determined the catalytic activity. The specific activity of purified recombinate OATase decreased 83.7% (from 86.44 to 14.13 Umg^{-1}) when glutamate was absent, suggesting that both the substrates were needed for OATase to synergistically exhibit *arg*A and *arg*E activities.

Separate Expression of Subunits from OATase

OATase from *C. crenatum* SYPA5-5 is a heterodimer which appears to be bifunctional. So we were interested in whether the α- and β-subunits could exhibit the enzymatic activity of AOase or NAGS, respectively, when synthesized independently. The *arg*J regions corresponding to the α- and β-subunits was cloned separately (Fig. 4), resulting in

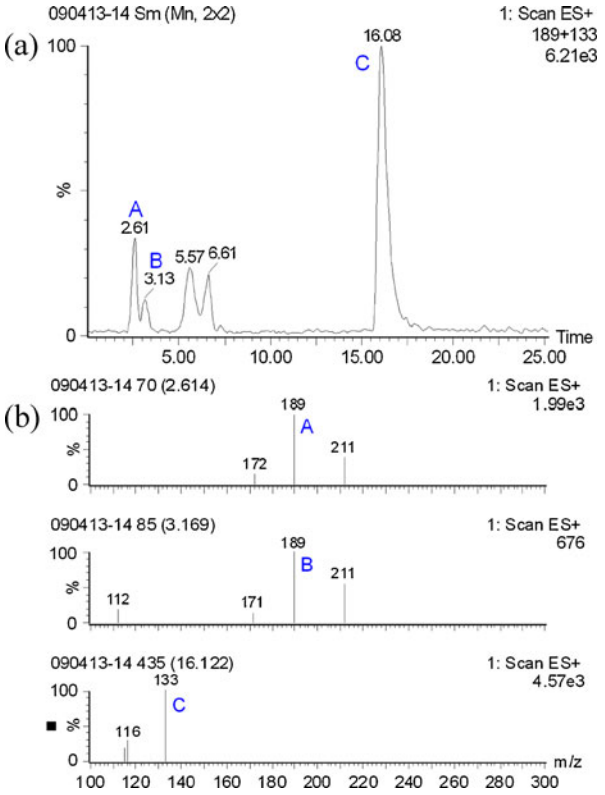

Fig. 3 OATase activity determined by LC-MS. *A*, *B* acetylglutamate. *C* ornithine. **I** LC analysis of OATase. **II** MS analysis of OATase

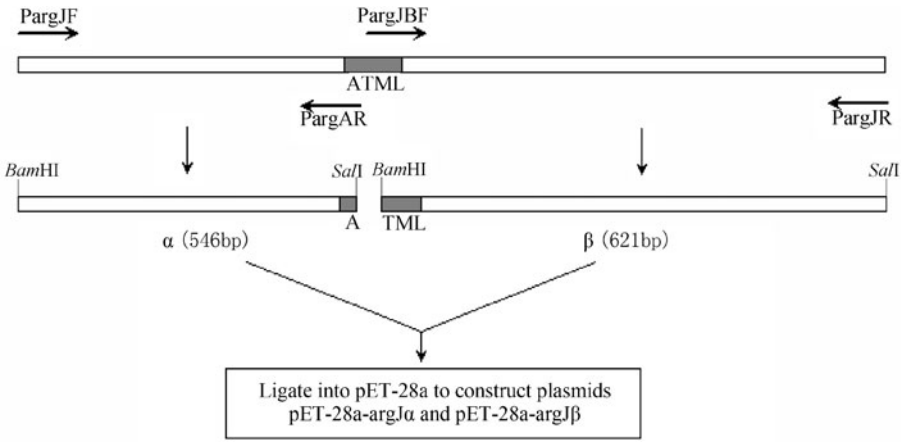

Fig. 4 Design of pET-28a-*arg*Jα and pET-28a-*arg*Jβ

recombinate *E. coli* BL21 (pET-28a-*arg*Jα) and *E. coli* BL21 (pET-28a-*arg*Jβ). As a result, the α- and β-subunits could not self-assemble to active OATase conformation. It is supposed that the α- and β-subunits must be combined together to play a role in the catalytic reaction, though there is no OATase activity observed when the α- and β-subunits were mixed in equimolar concentration after 30 min of incubation at 30°C.

Overexpression of the *arg*J Gene and its Effects on L-arginine Accumulation

To strengthen the overexpression of *arg*J in *C. crenatum*, the recombinant strain *C. crenatum* harboring plasmid pJC*tac-arg*J was constructed. Accordingly, the enzymatic activity of OATase in the culture supernatant of *C. crenatum* (pJC*tac-arg*J), which was treated by sonication, increased in comparison with the OATase activity in the supernatant of *C. crenatum* SYPA5-5. The results showed that OATase from *C. crenatum* (pJC*tac-arg*J) and *C. crenatum* SYPA5-5 had OATase activities in the level of 91.9 and 48.1 Ug^{-1}, respectively. Clearly, the OATase activity of recombinant strain was enhanced by 90.9%.

It has been reported that *arg*J played a vital role in the L-arginine biosynthetic pathway. Whether the overexpression of *arg*J would increase L-arginine accumulation in *C. crenatum* SYPA5-5 was studied. So the strains *C. crenatum* (pJC*tac-arg*J) and *C. crenatum* SYPA5-5 were fermented in a 5-L bioreactor using start strain *C. crenatum* SYPA5-5 as a control. The fermentation curves including concentrations of cells, glucose and L-arginine, the rate of growth and L-arginine production, as well as the yield of L-arginine ($Y_{P/X}$) are shown in Fig. 5. L-Arginine accumulated up to about 42.4 gL^{-1} in the culture medium, with a maximum specific productivity of 0.79 gL^{-1} h^{-1} at 48 h in *C. crenatum* (pJC*tac-arg*J) compared with 36.3 g L^{-1} and 0.63 gL^{-1} h^{-1} in the start strain, respectively (Fig. 5b). A comparison of the growth rates of the genetically modified strain with that of the start strain revealed that the overexpression of the gene *arg*J resulted in a decreased growth rate (Fig. 5a). The glucose consumption rate was also decelerated, which may be due to the decreased growth rate (Fig. 5c). In addition, the calculative $Y_{P/S}$ increased from 24.4% to 29.2%. The increase of the final L-arginine concentration and $Y_{P/X}$ in *C. crenatum* (pJC*tac-arg*J) further supported the fact that overexpression of *arg*J enhanced the metabolic flux of the L-arginine biosynthetic pathway.

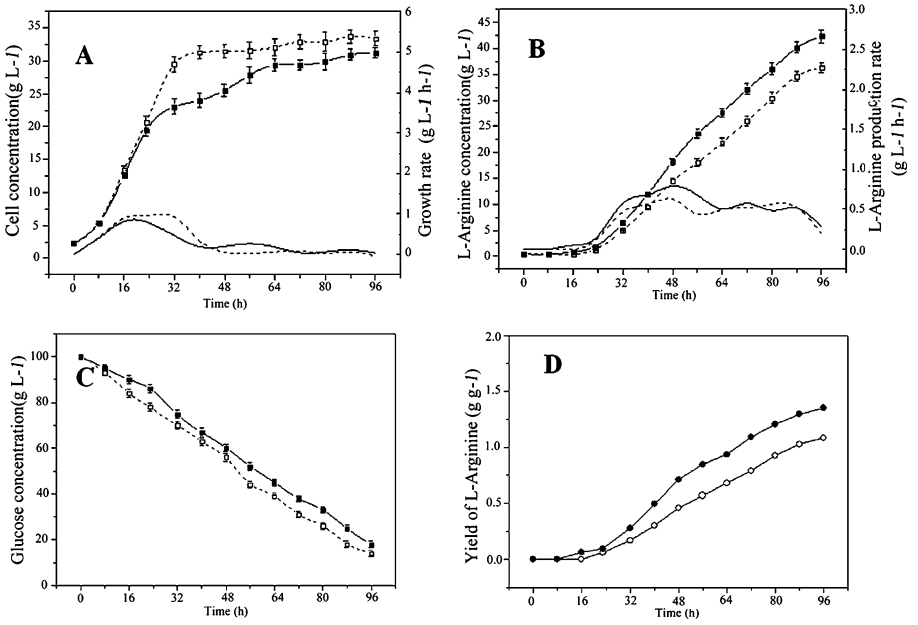

Fig. 5 Fermentation kinetics of *C. crenatum* (pJC*tac-arg*J) and *C. crenatum* SYPA5-5 in a 5-L bioreactor. **a** Cell concentration and growth rate of *C. crenatum*. **b** L-Arginine concentration and L-arginine production rate. **c** Glcucose concentration. **d** Yield of L-arginine. *Black line*/*black square*, *black circle*, *C. crenatum* (pJC*tac-arg*J); *broken line*/*white square*, *white circle*, *C. crenatum* SYPA5-5

Discussion

Our previous work had revealed that the 16SrDNA sequence similarity and the functional gene sequences homology of *C. crenatum* and *C. glutamicum* were very high (more than 99%). The genome sequence of *C. glutamicum* has been completed in 2003 [24]. Therefore, it is reasonable to consider using *C. glutamicum* ATCC13032 *arg*J as the model for the amplification and analysis of *C. crenatum* SYPA5-5 *arg*J. Sakanyan et al. [11] proposed that *arg*J in *C. glutamicum* codes a monofunctional ornithine acetyltransferase, while data in GenBank and ExPASy show that OATase in *C. glutamicum* ATCC13032 acts as a bifunctional enzyme [4, 25]. For the high homology between *C. crenatum* and *C. glutamicum*, we are interested in the real function of *C. crenatum* SYPA5-5 OATase in the L-arginine biosynthetic pathway.

In this study, the function of the OATase (*arg*J) of *C. crenatum* SYPA5-5 has been identified. When induced at a temperature lower than 16°C, OATase exists as two soluble subunits, which were folded exactly and cleaved in the ATML conserved sequence by autolysis of the recombinant OATase in *E. coli* at the low-temperature condition, whereas Abadjieva et al. [26] observed that the α- and β-subunits of *S. cerevisiae* OATase still exhibited a weak OATase activity when cloned and expressed separately. In this study, when the α- and β-subunits were cloned and expressed separately, the OATase, AOase, and NAGS activities were abolished. It was implied that self-catalyzed precursor cleavage is a necessary step to form active OATase.

The crude OATase-specific activity of *C. crenatum* SYPA5-5 was higher than that of *C. glutamicum* ATCC13032 by 67.4% (48.1 and 28.8 Ug^{-1}). It is possible that *C. crenatum*

SYPA5-5 could accumulate L-arginine as the difference of OATase activity between *C. crenatum* SYPA5-5 and *C. glutamicum* ATCC13032. In the subsequent expression of OATase in *E. coli* BL21 (DE3), we found that there was no significant difference in the specific activity of purified recombinant OATase from these two strains (86.44 and 85.30 U mg^{-1}, respectively). According to these results, we tentatively supposed that the high activity of OATase in *C. crenatum* SYPA5-5 was due to some other factors caused by the mutation instead of the difference of three amino acid residues in peptide chain.

Furthermore, *arg*J was overexpressed in *C. crenatum* SYPA5-5 and the content of L-arginine in *C. crenatum* (pJC*tac-arg*J) was increased by 16.8%. The overexpression of *arg*J indeed enhanced the metabolic flux of the L-arginine biosynthetic pathway. The proteomics analysis of several L-arginine-producing strains revealed that the expression of *arg*J was increased in the L-arginine hyper-producing strain (data not shown). In addition, the *C. glutamicum* argJ gene-disrupted had been constructed and the results indicated that an *arg*J-deficient arginine auxotrophic mutant of *C. glutamicum* was suppressed [18]. Meanwhile, the role of the α- and β-subunits in OATase is currently under investigation in our laboratory. The study of the *arg*J gene is certainly beneficial to further modify *C. crenatum* SYPA5-5 metabolism in breeding more excellent L-arginine producers.

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