

# 論文内容要旨

Development of a system of high ornithine and  
citrulline production by a plant-derived lactic acid  
bacterium, *Weissella confusa* K-28

(植物由来乳酸菌 *Weissella confusa* K-28 における  
オルニチン及びシトルリン高生産システムの構築)

Biological and Pharmaceutical Bulletin, 2019, in press.

主指導教員：杉山 政則 共同研究講座教授

(医系科学研究科 未病・予防医学)

副指導教員：小池 透 教授

(医系科学研究科 医薬分子機能科学)

副指導教員：野田 正文 特任准教授

(医系科学研究科 未病・予防医学)

MD RAKHIMUZZAMAN

(医歯薬保健学研究科 薬科学専攻)

## **Introduction**

Probiotics are defined as live microorganisms that, “when administered in adequate amounts, confer a health benefit on the host.” Lactic acid bacteria (LAB) are the typical probiotics, non-pathogenic, and generally recognized as safe (GRAS). It has been reported that LAB contribute to health-promotion, such as immunomodulation, reduction of obesity, and anti-allergic inflammation in human.

In the present study, the author has successfully isolated a plant-derived lactic acid bacterium, designated K-28, and identified it as *Weissella (W.) confusa*. As a result, the author has found that the strain produces ornithine at a high level.

Ornithine is non-protein amino acid and provides healthcare benefits including liver protection, because the amino acid is a metabolic intermediate that plays a role in the urea cycle by detoxifying the ammonia to reduce the level of ammonia in the blood.

An aim of this study is to establish the culture condition to produce ornithine at a high level together with citrulline.

## **Results**

### **1. Isolation and identification of ornithine-producing LAB**

Twenty LAB strains were isolated from several plants. As a result, a LAB strain designated K-28, which was isolated from the flower of *Senna obtusifolia*, was found to produce a large amount of ornithine in the MRS medium supplemented with arginine. The entire 16S rDNA sequence of the strain showed that the K-28 strain was identified as *Weissella (W.) confusa*.

### **2. Culture conditions for high-yield ornithine production**

In the present study, the author has evaluated the effects of initial pH of the medium (4.5–9.0 at 0.5 intervals) to produce ornithine at a high level. The result shows that the initial pH for the efficient production is between pH 5.0 and 8.0. The culture temperature and the harvest period to enhance ornithine production have been evaluated, suggesting that the cultivation at 28°C is better for the cell growth and the high-level production of ornithine. In this case, the cultivation for 48 h is suitable to increase the ornithine productivity. In addition, it has been observed that high concentration of arginine added to the medium gives rise to the saturation of arginine conversion to ornithine whereas low arginine concentration (0.5% w/v) increases the conversion ratio from arginine to ornithine.

### **3. Effect of fed-batch fermentation on high-yield ornithine production**

The author has expected that the K-28 strain produces a large amount ornithine by the fed-batch culture supplemented with arginine. For the cultivation test at a jar-fermenter scale, the K-28 strain was grown in 1 L of MRS medium supplemented with 0.5% (w/v) arginine by maintaining the pH at 6.5 using a 3 L jar fermenter with agitation at 28°C for 96 h. Each 12 h interval, 10% (w/v) [final 0.5% (w/v)] arginine containing fresh MRS medium (pH 6.5) was added and sampling was continued to the fermenter for 96 h.

The arginine conversion to ornithine drastically increased to over 100%. Finally, the production of ornithine and citrulline by the strain was  $18 \pm 1$  g/L and  $10 \pm 2$  g/L, respectively, with a conversion ratio  $100 \pm 9\%$ .

#### **4. Gene organization and Expression Analysis of the ADI Gene Cluster**

To characterize the arginine deiminase (ADI) gene cluster of the high-ornithine-producing *W. confusa* K-28, the author has determined the gene organization of the cluster, which is 5.7 kb long, consists of five genes.

The author has evaluated the expression of each gene in the ADI gene cluster during the fed-batch culture using a jar fermenter and the RT-PCR method. The result shows that the expression level of the genes decreases gradually with the cultivation time. The gene expression of the putative regulator *wkaR* also decreases gradually with the cultivation period, suggesting that the *wkaR* product may function as a positive regulator of ornithine production.

#### **5. Ornithine Production under the Aerobic or Anaerobic Condition**

The expression level of each gene in the ADI cluster under the aerobic or anaerobic condition was evaluated. The RT-PCR analysis shows that the expression of five genes in the ADI gene cluster is higher under the anaerobic condition than under the aerobic one. The result suggests that ornithine production was significantly high under the anaerobic condition ( $20.2 \pm 0.3$  mM) at 24 h of cultivation but not under the aerobic condition ( $4.1 \pm 0.4$  mM ornithine).

### **Discussion**

The production of ornithine by the microbial fermentation still is not to an industrially satisfactory level. The limited strains, which is a *C. glutamicum* mutant strain bred by the mutagenesis, have been evaluated to produce a large amount of ornithine. In contrast to the mutant strain, the wild-type *C. glutamicum* produces only 0.5 g/L ornithine. The present study shows that *W. confusa* K-28, which harbors the ADI pathway, has significant potential to produce ornithine at a high level. In fact, it has been observed that the strain produces a high amount of ornithine ( $67 \pm 4$  mM) *via* a continuous arginine-feeding method for 24 h. The production is five-fold higher than that *via* the stand-cultivation method ( $12.7 \pm 0.6$  mM) for 24 h.

Arginine catabolism *via* the ADI pathway has been reported in some LABs and other bacteria. The author has compared the arginine catabolism and ornithine production by *W. confusa* K-28 with that by other strains. A research group has reported that *W. koreensis* MS1-3 and *W. koreensis* MS1-14 produced extracellular ornithine at 45 mg/L and 46 mg/L respectively, when arginine was supplemented with a final concentration of 1.0% (w/v). However, *W. confusa* K-28 produced 8.85 g/L ornithine under the same culture conditions.

Through an acute toxicity test and a mutagenicity experiment, *W. confusa* K-28 has been confirmed to be a safe and reliable bacterium. Therefore, the K-28 strain may be useful as a potential probiotic for manufacturing functional food and healthcare supplements.

### **Reference**

This study is currently being submitted to an international journal.