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Changing industrial waste into healthy ingredients−Generating amino acids from soy pulp

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The purpose of this study was to devise a method for recycling soy pulp from Okinawan tofu industrial waste and to contribute to the extension of healthy life expectancy in Japan. This study focused on the amino acid-producing ability of lactic acid bacteria. Lactic acid bacteria were isolated from Okinawan tofu soy pulp and were screened for useful strains that indicated amino acid production capacity. We succeeded in isolating a *Lactococcus lactis* strain that produces isoleucine abundantly as an essential amino acid from soy pulp. In the future, soy pulp should be commercially produced using the strain identified in the present study to enhance the strength of the elderly.

Keywords: industrial waste, soy pulp, recycling, amino acid, lactic acid bacteria

1. Introduction

1.1 The problem in Okinawa

1.1.1 To spend the latter period of a more substantial life

The residents of Okinawa prefecture have historically been known to have good longevity. Ogimi-son in Okinawa is a particularly famous village with regard to longevity and the eating habits of the residents have been widely adopted. However, recently, the impression of Okinawa as a longevity prefecture is fading. According to data from the Ministry of Health, Labour and Welfare (2015), women and men from Okinawa ranked nationally 7th and 36th, respectively, in terms of length of the average life span, demonstrating that neither group lived particularly long despite Okinawa's reputation as a longevity prefecture. A decrease in life expectancy is considered to be a problem that should concern healthcare professionals. Healthy life expectancy is the period of time that one can live with a healthy self-supporting body and mind. Recently, the difference between life expectancy and healthy life expectancy in Japan has become an important issue. The number of elderly people in Okinawa Prefecture is estimated at 11.3% of the population, and this number is increasing. Therefore, in view of the rising proportion of elderly citizens, prolonging the healthy life expectancy, for example, do not be lived a life of the bedridden old age, has become a pressing issue in Okinawa Prefecture.

1.1.2 Treatment sites of industrial waste

Issues related to industrial waste processing have become a problem in Okinawa Prefecture and similar regions. Since Okinawa consists mainly of islands, many restrictions exist regarding the disposal of industrial waste, limiting the disposal capacity of the islands. In an environment such as Okinawa, the processing of the soy pulp used to produce tofu represents a major industrial waste problem. Island tofu, made using the original Okinawa manufacturing method, is a traditional food that is indispensable to local cooking. However, approximately 5,000 tons of island soy pulp is produced during the manufacturing process annually, representing approximately 500 million yen in processing expenses. In Okinawa, limited processing space necessitates the development of novel disposal methods for this and other industrial wastes.

1.2 The proposed solution

In the present study, we aimed to address two problems: to produce amino acids by lactic acid bacteria and to establish a novel disposal method for soy pulp. As a solution to these problems, we aimed to develop a food product using soy pulp that could support a healthy lifestyle for the elderly. In this approach, we used a lactic acid bacterium to ferment soy pulp and increase the amino acid content, especially that of the BCAAs (branched-chain amino acid ; valine, leucine, and isoleucine)[1)], which are metabolic products of fermentation. By producing amino acids that are useful for health management and having them consumed by the elderly, it is possible to extend the healthy life span of the elderly.

2. Materials and methods

2.1 Isolation of lactic acid bacteria from island soy pulp

Experiments were conducted at the Research and Education Center for Subtropical Resources at the National Institute of Technology, Okinawa College; soy pulp was allowed to stand for 72 h. A standing petri dish was used to mix 10 g of soy pulp and 5 mL of water. After collection, the mixture was suspended in purified water and was spread over MRS (MRS: de Man, Rogosa, Sharpe) agar medium.

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After 24 h of storage at 37°C, emerging colonies were observed on the agar. The strains were subsequently isolated and cultured in MRS liquid medium. A total of 94 strains were eventually isolated.

2.2 Selection of promising strains based on their BCAA-producing ability

The BCAA content of the lactic acid bacterial cultures of the isolated 94 strains was analyzed using Liquid Chromatography-Mass Spectrometry (LC/MS). The analysis conformed to the APDSTAG Wako (FUJIFILM Wako Pure Chemical Corporation) protocol[2].

2.3 Genus identification of the promising stocks

The genera were identified using the Sanger method with a capillary sequencer, employing 16s rRNA analysis.

2.4 Culture condition optimization

BCAA production of the lactic acid bacteria was studied under various culture conditions to determine the optimal conditions for maximum output. First, the proportion of the culture solution, water, and soy pulp was optimized in a test tube to 1:1:10. Fermentation time was also optimized using observations under five test conditions: 24, 48, 72, 96, and 120 h. After completion of fermentation, the culture solution was filtered to measure BCAA content via LC/MS. The number of lactic acid bacteria at the beginning of fermentation was approximately 1.0×10^6 cells, which was held constant until the end of fermentation, at which time it was measured again to calculate the number of lactic acid bacteria at the end time point.

3. Results

3.1 Selection of promising strains based on their BCAA-producing ability

Using LC/MS, the amino acid content in the culture broth of the 94 strains of lactic acid bacteria isolated from the soy pulp was measured. The detected values of BCAA measured by LC/MS were shown in Table 1. Based on the results, the three strains with the highest BCAA production level were selected as promising strains. The LC-Ikematsu strain held in the laboratory was used as a control for measurement. This sample is known to produce BCAA in previous studies. Similarly, the average value of all samples and the value of uncultured samples are shown for comparison.

Table 1 BCAA production of the three most productive strains obtained after normalization to a blank.

Strain	Valine (μM)	Isoleucine (μM)	Leucine (μM)
No. 15	86.4	24.9	95.3
No. 40	129.4	30.6	228
No. 57	99.5	33.8	133.1
control	N.D.	N.D.	N.D.
average value of the all samples	35.3	8.4	46.8
without fermentation (initial)	19.1	4.5	40.4

3.2 Genus identification of the promising strains

The genera of the selected strains were identified using the Sanger method and were listed in Table 2. The homology search was performed using BLAST.

Table 2 Results of the genus identification test using the Sanger method

Strain	Genus	Identities (%)
No. 15	*Lactococcus garvieae*	99
No. 40	*Lactococcus lactis*	99
No. 57	*Lactococcus garvieae*	98

3.3 Optimization of culture conditions

The fermentation time yielding optimal BCAA productivity was identified by examining the amount of BCAAs produced and the count of lactic acid bacteria following varying fermentation times. Each sample was measured three times (n=3). The bars showed the bacteria count. And polygonal line shows BCAA concentration. A square marker indicates valine, a triangular marker indicates isoleucine, and a round marker indicates leucine. The details were shown in Fig. 1, Fig. 2 and Fig. 3.

4. Discussion

- The LC-Ikematsu strain showed an increased number of bacteria with increasing culture time, but the amino acid content decreased (data not shown).
- For strain No. 40, with increasing incubation time, the amount of isoleucine produced per bacterium increased. Therefore, it was suggested that strain No. 40 showed improved amino acid productivity with extended incubation time. Based on this result, strain No. 40 showed great promise in light of the possibility of future commercialization.
- In strains No. 15 and No. 57, leucine content was high in the 48 h culture, but with increasing culture time, the amount of amino acids decreased despite the increase in the number of bacteria, confirming lowered productivity of these strains. Moreover, it should be noted that the overall trend for strains No. 15 and No. 57 was similar because they originated from the same genus.

5. Conclusions

We found three lactic acid bacteria strains that produce useful amino acids using lactic acid bacteria fermentation from soy pulp destined to become industrial waste. Two of the three strains isolated were *Lactococcus garvieae*, and the remaining strain was *L. lactis*. The latter was found to produce a large amount of isoleucine via soy pulp fermentation; isoleucine is an essential amino acid that is

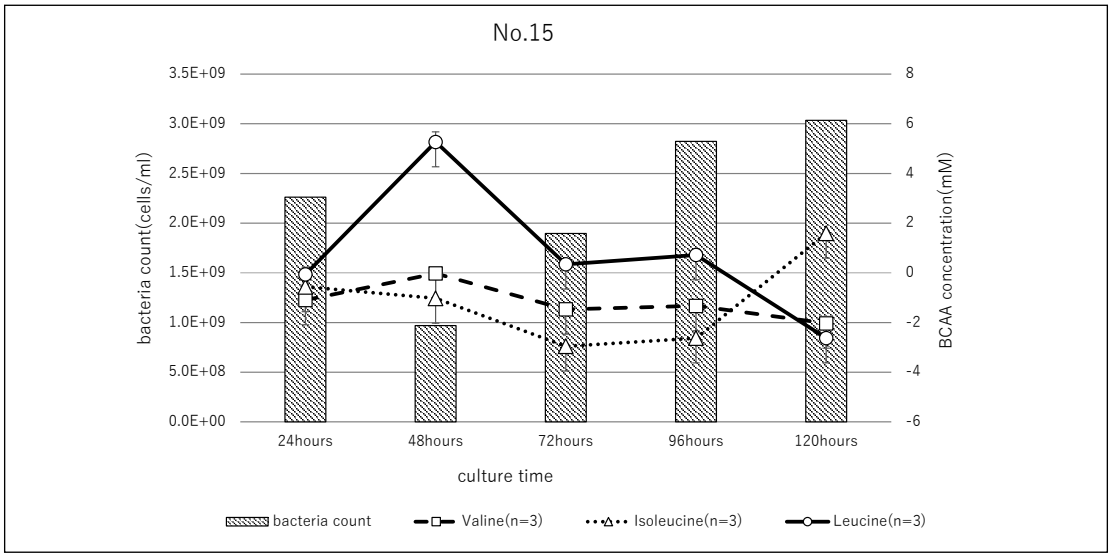

Fig. 1 BCAA content (polygonal line) and number of bacteria (bars) at various incubation times for strain No. 15.

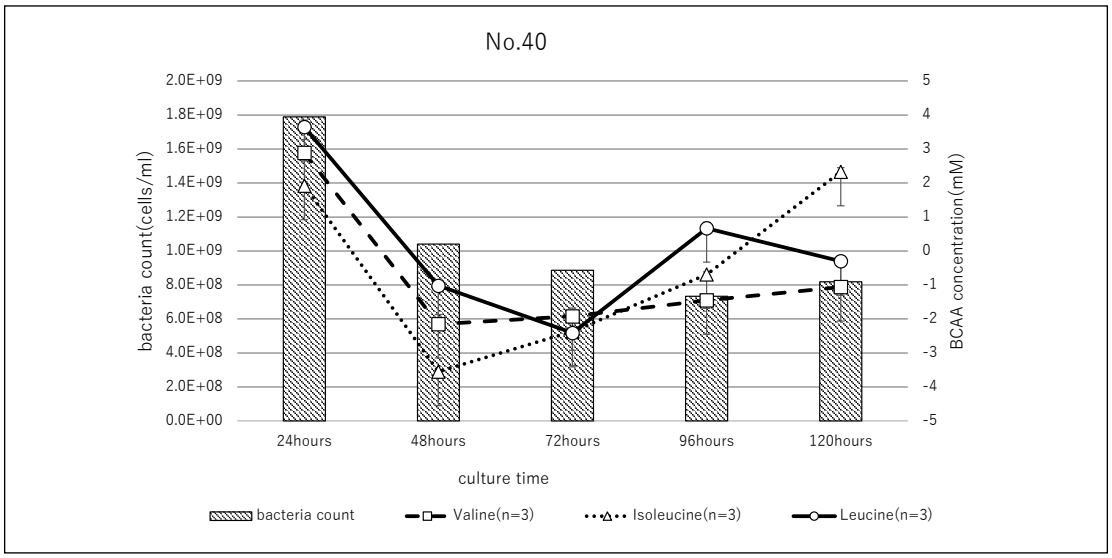

Fig. 2 BCAA content (polygonal line) and number of bacteria (bars) at various incubation times for strain No. 40.

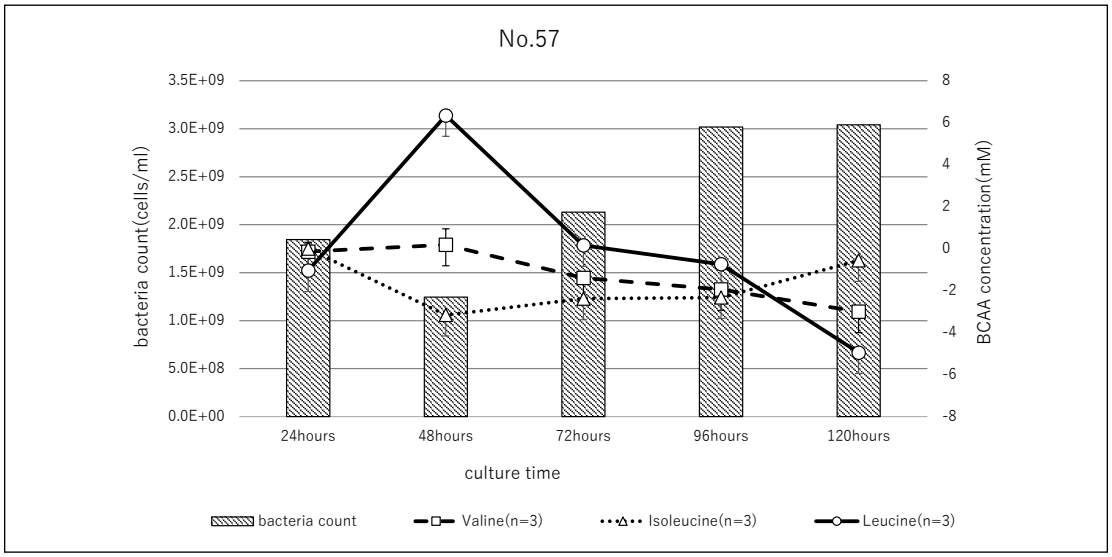

Fig. 3 BCAA content (polygonal line) and number of bacteria (bars) at various incubation times for strain No. 57.

effective in muscle building. In Japan, a continuing transition to an aging society is predicted for the future, with Okinawa being a typical example. However, the current research results will possibly contribute to extension of the healthy life of the elderly in terms of physical condition management based on muscle strength maintenance.

6. Future outlook

- Whole-genome sequencing of the isolated bacteria and analysis of single points on the genome.
- Reexamination of fermentation conditions to establish optimal conditions for BCAA production.
- Setting of appropriate positive controls and clarifying standard BCAA content.
- Extraction of these BCAAs from bacterial cells given the possibility of BCAA accumulation in the bacteria.

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