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Purification and Identification of 1-Deoxynojirimycin (DNJ) in Okara Fermented by *Bacillus subtilis* B2 from Chinese Traditional Food (Meitaoza)

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This study was to purify an α-glucosidase inhibitor from okara (soy pulp) fermented by *Bacillus subtilis* B2 and to identify its chemical structure. Membrane dialysis, active charcoal, CM-Sepharose chromatography, and preparative thin-layer chromatography (TLC) were used in the purification, while positive mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectrometry were used in the identification. The MS and NMR data showed that the purified α-glucosidase inhibitor was 1-deoxynojirimycin (DNJ) with a molecular weight of 163 Da. This is the first time that DNJ was isolated from foods fermented with *Bacillus* species. Okara fermentation with *B. subtilis* B2 might be used to produce a food-derived DNJ product as a functional food for diabetic patients.

KEYWORDS: α-Glucosidase inhibitor; *Bacillus subtilis* B2; purification; fermented okara; 1-deoxynojirimycin

INTRODUCTION

1-Deoxynojirimycin (DNJ), a typical naturally occurring azasugar, is a D-glucose analogue with an NH-group substituting for the oxygen atom of the pyranose ring (*1*). Substituting with an alkaloid component is one of the therapeutic approaches for reducing postprandial hyperglycemia by inhibiting α-glucosidase activity and thereby reducing the absorption of dietary carbohydrates (*2*−*4*). DNJ and some of its derivatives have been demonstrated to be effective α-glucosidase inhibitors, with promising biological activities, such as potential antidiabetic, antitumor, and anti-HIV activities (*5*). For example, miglitol, a DNJ derivative, was introduced onto the market by the U.S. Food and Drug Administration (FDA) in 1999 as a more potent second-generation α-glucosidase inhibitor with fewer gastrointestinal side effects.

DNJ was first obtained from the antibiotic nojirimycin by catalytic hydrogenation (*6*) and later isolated from the roots of mulberry trees (*2*). DNJ is also produced by several *Bacillus* and *Streptomyces* species (*7*) isolated from the soil. In recent years, scientific evidence has demonstrated that the morbidity and mortality of diabetes can be reduced by aggressive treatment with diet and exercise to achieve better control of blood glucose levels, which gave rise to increased interest in foods containing the active compound DNJ (*8*). Mulberry leaves have been known to be rich in DNJ, which is believed to be beneficial for suppressing abnormally high blood glucose levels as a dietary food (*9*, *10*). At present, various food grade mulberry products (such as teas, powders, and tablets) have been made commercially available in Japan and many other countries. This evidence shows that the intake of food-derived DNJ in our foods may be an attractive therapeutic approach for treating postprandial hyperglycemia with an improved safety profile.

Nevertheless, the production of food-derived DNJ products from mulberry usually is limited by several factors. The DNJ content in commercial preparations of natural mulberry leaf is as low as 0.1% (*10*), which may be too low to show an effect (*11*). Furthermore, the DNJ content in mulberry generally depends on a variety of factors such as the growth season, the age of the leaves, and the cultivated areas, which affect the amount of DNJ in different batches of the same product. The variation in the DNJ content of mulberry stuff will increase the difficulty of controlling the constant DNJ levels in the final products although the fluctuation of the DNJ levels might be minimized by quality assurance. Third, the cost of mulberry products is high due to the length of their growth period and the cultivation. Food derived from fast-growing microorganisms represents an attractive alternative to the mulberry products. Although several researchers have reported that species of *Bacillus* and *Streptomyces* (*7*, *12*) were able to synthesize DNJ, all of these microorganisms were isolated from soil, which was difficult to be

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directly used in the production of the food-derived DNJ products. Thus, there is much interest in identifying a microorganism of suitable origin for the production of foods capable of producing DNJ.

In the previous study, we isolated food origin *B. subtilis* B2 capable of enhancing the antioxidative activity and the α-glucosidase inhibitory activity of fermented okara (*13*−*15*). However, the active compounds inhibiting the α-glucosidase activity are not yet clear. In this paper, we tried to identify the active component from Chinese traditional fermented okara (soy pulp).

MATERIALS AND METHODS

Microorganism. *B. subtilis* B2 was isolated from Meitauza (a Chinese traditional fermented okara) in a previous study and identified by the Institute of Microbiology of the Chinese Academy of Sciences (Beijing, China) using 16S rDNA polymerase chain reaction−restriction fragment length polymorphism (PCR-RFLP). The bacteria culture was maintained on a nutrient agar slant (Oxoid) and stored at 4 °C. The culture was inoculated into 30 mL of Luria−Bertani (LB) medium (Oxoid) and allowed to grow at 40 °C for 16 h. The enriched culture was diluted with sterile distilled water containing 0.9% NaCl and 0.1% peptone to prepare a culture suspension of approximately 10^6 colony forming units (cfu)/mL and served as inocula culture for the fermentation of okara.

Preparation of Okara. Soybeans (2000 g) harvested in 2006 at the Center of Soybean Research, Agricultural Academy of Jilin Province (Jilin, China), were soaked in distilled water (1:3 w/w) at room temperature for 12 h, which brought the soaked soybeans to approximately 2.2 times their original weight. The soaked soybeans were used to produce fresh okara according to the modified method of Shih, Hou, and Chang (*16*). Hydrated soybeans were ground and filtered in a high-speed grinder with distilled water (1:7 w/w). Fresh okara (85% moisture) was obtained after removal of the liquid containing the water-soluble components from soybean.

Fermentation of Okara. Distilled water was added to the fresh okara until the concentration of okara in the growth medium reached 4.5%. The growth medium (30 mL) containing 4.5% of okara was placed in a 150-mL Erlenmeyer flask and sterilized at 121 °C for 20 min in an autoclave. Sterilized growth medium was inoculated with 1% (v/v) of the *B. subtilis* culture suspension and incubated at 40 °C with shaking at 150 rpm for 96 h. Four milliliters of the culture broth was taken from the flask after incubating for 4, 12, 24, 48, 72, and 96 h. For analysis of α-glucosidase inhibitor activity, the culture broth was centrifuged at 3000*g* for 15 min at 4 °C and the supernatant was filtered through a 0.45-μm membrane under vacuum (Millex-HX, Millipore, MA). The filtrate was collected as fermentation broth and analyzed for α-glucosidase inhibitory activity.

In order to prepare a large amount of sample for the purification and identification of the α-glucosidase inhibitor, one liter of sterilized growth medium containing 4.5% okara was prepared and fermented for 72 h at 40 °C with shaking at 150 rpm. After that, the fermented liquid was centrifuged at 3000*g* for 15 min at 4 °C and the supernatant was filtered through a 0.45-μm membrane under vacuum (Millex-HX, Millipore, MA). The filtrate was immediately dried in a freeze-dryer (FDU-54, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) for further purification and analysis.

Determination of α-Glucosidase Inhibitory Activity. The inhibitory activity of the fermentation broth against α-glucosidase was determined by reaction between α-glucosidase and 4-nitrophenyl α-D-glucopyranoside (4-NPG, Sigma Chemical Co., St. Louis, MO) according to the protocol using a multiwell plate (96 wells flat bottom, Sumitomo Bakelite Co., Ltd., Tokyo, Japan) as described by Yamaki and Mori (*17*). The fermentation broth was serially diluted with an equal volume of distilled water and dispensed into wells of the plates (20 μL per well) followed by the addition of 50 μL of suspension of rat intestine acetone powder (25 mg/mL, Sigma Chemical Co., St. Louis, MO), 50 μL of 4-NPG (3 mM) as substrate, and 120 μL of 0.5 M phosphate buffer (pH 6.7). The mixture was incubated at 37 °C for 45 min to allow α-glucosidase to react with 4-NPG and produce 4-nitrophenol. The reaction was terminated with the addition of sodium carbonate (50 μL, 0.67 M). Formation of 4-nitrophenol in each well was measured by the intensity of absorbance at 405 nm using a microplate reader (BIO-RAD Lab. Model 550, CA). The α-glucosidase inhibitory activity of the fermentation broth was then calculated as the slope of the curve of absorbance versus concentration of the fermentation broth. A higher slope indicates stronger α-glucosidase inhibitory activity of the fermentation broth.

Purification of the α-Glucosidase Inhibitor. *Ethanol Precipitation*. The lyophilized powder of fermented liquid was dissolved in 10 mL of distilled water, adjusting the ethanol concentration to 80% with ethanol. In order to precipitate the polysaccharide and protein sufficiently, the 80% ethanol solution of the sample was placed in a refrigerator at 4 °C for 1 h. After centrifugation, the corresponding supernatant was concentrated to 5 mL by vacuum evaporation and then immediately freeze-dried (FDU-54, Tokyo Rikakikai Co., Ltd.) to obtain the lyophilized powder of sample after ethanol precipitation for the next purification step.

Dialysis. Two types of dialysis membranes were used for the purification: 100 Da (Da) and 1000 Da molecular weight cutoffs (MWCO) (Spectra/Por* Biotech Cellulose Ester (CE) Dialysis Membrane Tubing, Spectrum Laboratories., Inc., USA). The lyophilized powder of sample after ethanol precipitation was dissolved in 5 mL of distilled water and filtered under a vacuum with a 0.45-μm membrane (Millex-HX, Millipore). The filtrate was dialyzed using the membrane with 100 Da MWCO, and the outside solution of the membrane was replaced at regular intervals of 2 h, 6 h, and 12 h. All the outside solution containing the compounds with molecular weight below 100 Da was collected and mixed and then concentrated to a lyophilized powder for analysis of the α-glucosidase inhibitory activity. The inside part of the dialysis membrane was transferred to the dialysis membrane with 1000 Da MWCO. The dialysis process was the same as above. All the outside solution and the inside solution of the membrane were collected separately and freeze-dried for further analysis and purification.

Active Charcoal Chromatography. The active charcoal (WAKO Pure Chemical Industries Ltd., Japan) was packed in a column (2.8 cm × 45 cm) with a final column volume of 240 mL. Before the sample was applied, the column was balanced with distilled water for 180 min at a flow rate of 1.5 mL/min. After the sample was applied, 5%−30% of ethanol solution−distilled water was used for the gradient wash at a flow rate of 1.5 mL/min. The flow-out solution was collected at 5-min intervals, and the α-glucosidase inhibitory activity of each collected part was analyzed. The parts with high α-glucosidase inhibitory activity were combined and freeze-dried for further CM-Sepharose chromatography.

CM-Sepharose Chromatography. CM-Sepharose chromatography was used for the further purification. Before the sample was applied to the large scale column (5 cm × 20 cm, Sigma-Aldrich Co.), the mini-CM-Sepharose column (5 mL) (HiTrap SP, GE Healthcare UK Ltd., England) was used to determine the elution conditions. The sample was dissolved in the formic acid solution (pH 3.7) in order to become positively charged. After applied the sample, the formic acid solutions with various pH values were used to elute the active compound. The elution was collected at regular intervals and dried using a vacuum freezing centrifugation dryer. The α-glucosidase inhibitory activity of each collected part was determined to identify the optimum pH of the formic acid solution for eluting the active compound.

After that, CM-Sepharose chromatography (5 cm × 20 cm, GE Healthcare UK Ltd.) was used to purify a larger amount of sample using the formic acid solution with fixed pH value at a flow rate of 5 mL/min. The elution with strong α-glucosidase inhibitory activity was combined and dried for further purification.

Preparative Thin-Layer Chromatography. The active sample was dissolved in a small amount of methanol−water solution (1:1) and spotted on the preparative thin-layer plates (Silica gel 60 F254, 2 mm, Merck Ltd., German). The plate was developed with three runs of chloroform−methanol−5.6% ammonium (30:30:10). The silica in the area with α-glucosidase inhibitory activity was collected, and the water was used to extract the active compound bond on the silica. The extracting water with active compound was immediately freeze-dried for further analysis.

Purity Determination. The purity of the active compound was determined by a high-performance liquid chromatography (HPLC) method. HPLC analysis was modified based on a method with an amide type column and an evaporative light-scattering detector (ELSD) (*9*). The TSK-Gel-amide-80 column (4.6 mm × 250 mm, 4 μm, Tosoh, Tokyo, Japan) was used in the Shimadzu LC-10A HPLC system. The separation was performed using a mixture of acetonitrile and distilled water (81:19, v/v, containing 6.5 mM ammonium acetate; pH 5.5). The flow rate was adjusted to 1 mL/min, and the column temperature was maintained at

70 °C. The eluent was split at the postcolumn. One of the split eluents was sent to a SEDEX 55 ELSD detector (Sedere, Alfortville, France). The conditions were optimized to achieve maximum sensitivity; the temperature of the drift tube was set at 80 °C, the nebulizing gas was at a pressure of 2.3 bar, and the gain was set at 8. The DNJ standard and the purified sample were weighed accurately and dissolved in a mixture of acetonitrile and water (50:50; containing 6.5 mM ammonium acetate; pH 5.5), and 20 μL samples of these solutions were subjected to the HPLC−ELSD system.

Identification of the Structure of the α-Glucosidase Inhibitor. *Positive Mass−Mass Spectrometry* (*MS−MS*). Mass detection and MSn experiments were performed on a ThermoFinnigan LCQclassic mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an ESI source and an ion trap mass analyzer. The mass range of *m*/*z* is from 50 to 2000. The whole system was controlled with Xcalibur software. The purified sample was dissolved in an acetonitrile/water solution (1:1, v/v). 20 μL of the sample solution was directly injected for the multiple stage (MS−MS) analyses. In the full scan mode, the mass spectrometer was operated over a range of *m*/*z* 50−500 in the positive mode. MS2 was performed in 37% of the collision energy of the parent ion (*m*/*z* 164), and MS3 was performed in 37% of the collision energy of the daughter ion (*m*/*z* 146). The standard DNJ was also analyzed by MSn under similar conditions with the sample.

Nuclear Magnetic Resonance (NMR) Spectrometry. The TLC fraction (1 mg) was dissolved in D_2O (0.75 mL), and nuclear magnetic resonance (NMR) spectra were measured at 298 K on an Avance 800 spectrometer (Bruker Biospin, Karlsruhe, Germany). To find the center position offsets for hydrogen and carbon to incorporate during processing of the sample spectra, the spectrum of 2,2-dimethylsilapentane-5-sulfonic acid (DSS) as an internal standard was performed at 298 K. The hydrogen offset was measured directly. The carbon offset was calculated indirectly from the hydrogen result. The active component of this fraction was identified using ^{1}H NMR and ^{13}C NMR. In order to compare, ^{1}H NMR and ^{13}C NMR of the standard DNJ were also performed. Furthermore, two-dimensional NMR experiments including double-quantum-filtered correlation spectroscopy (DQF-COSY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC) were also performed to obtain the structure of the sample.

RESULTS AND DISCUSSION

α-Glucosidase Inhibitory Activity of Fermented Okara. Our previous study showed that the fermentation broth of *B. subtilis* B2 in Luria−Bertani (LB) with okara as the additional nitrogen source exhibited strong α-glucosidase inhibitory activity, which might be considered as a strategy for preparing functional foods for diabetic patients (*15*). Nevertheless, the higher cost of the LB culture than okara led us to pursue a more cost-effective culture with similarly high α-glucosidase inhibitory activity. As the content of protein, oligosaccharide, and fiber in okara is rich, okara was used as a unique culture to cultivate the *B. subtilis* B2 to produce α-glucosidase inhibitory activity in our current study. The α-glucosidase inhibitory activity of the fermented okara broth under various fermentation times is shown in **Figure 1**. The initial unfermented okara exhibited a very low α-glucosidase inhibitory activity of 0.33 (slope value), while the activity of the fermented okara broth increased gradually with longer fermentation time. The α-glucosidase inhibitory activity increased drastically from 0.33 up to 22.8 (slope value) during the first 48 h of fermentation and thereafter increased slightly from 48 to 96 h (end of the fermentation) when it reached 24.3 (slope value). Taking both the cost of fermentation and the α-glucosidase inhibitory activity into account, 48 h of fermentation time was selected for the further large-scale preparation of the α-glucosidase inhibitor. These data indicate okara appears to be a potential candidate for the production of α-glucosidase inhibitor by *B. subtilis* B2.

It has been suggested that the α-glucosidase inhibitors can be synthesized by chemical methods (*18−20*) or be isolated naturally from plants or food products (*21−23*). Nevertheless, these chemically synthesized α-glucosidase inhibitors normally cause hepatic disorders and other negative gastrointestinal symptoms due to their strong inhibitory effect, metabolism patterns, or

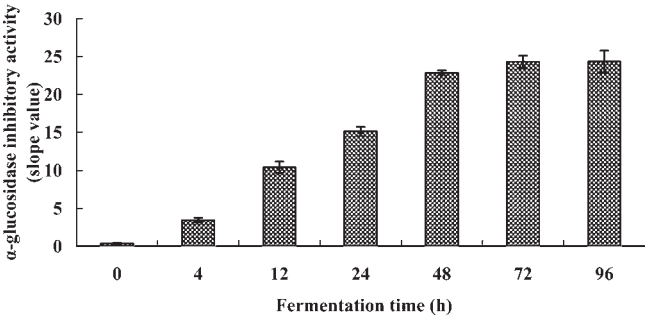

Figure 1. α-Glucosidase inhibitory activity of fermented okara broth. Values represent the means + standard deviation (SD) of $n = 3$ duplicate assays of one fermentation.

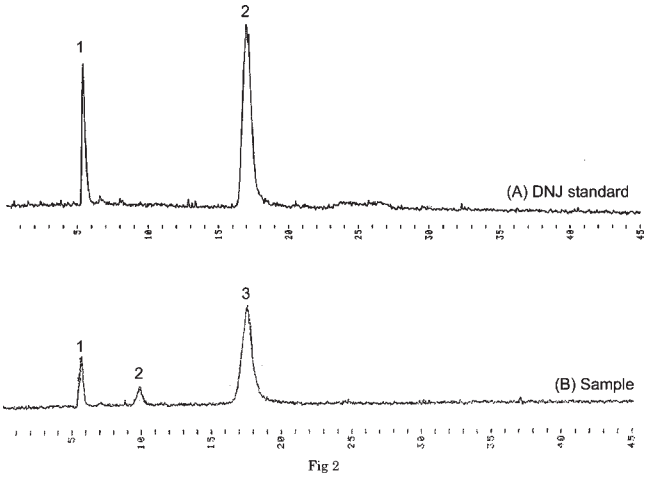

Figure 2. HPLC chromatograph using ELSD detection.

(A) HPLC chromatogram of DNJ reference standard. Peak 1 is the solvent peak; peak 2 is standard DNJ. (B) HPLC chromatogram of active component. Peak 1 is the solvent peak; peak 2 is unknown compound; peak 3 is the major compound in the sample.

Table 1. Summary of the Purification of the α-Glucosidase Inhibitor

purified fraction	weight (mg)	IC_{50}[a] (μg/mL)	DNJ eq[b] (mg)	recovery[c] (%)
freeze-dried broth	3000	73.5	2.20	100
ethanol precipitation (Supernatant)	1500	40.9	1.83	83.3
dialysis (part II)	400	13	1.54	69.9
activated charcoal fraction	200	8	1.25	56.8
CM sepharose fraction	5	0.24	1.04	47.3
preparative TLC fraction	3.55	0.2	0.89	40.3

[a] IC_{50} = concentration at which 50% inhibition occurs. [b] DNJ eq = (weight of the sample/IC_{50} of the sample) × IC_{50} of DNJ. [c] Recovery (%): Taking the recovery of the freeze-dried powder of fermentation broth as 100, the recovery of each step is calculated as: DNJ eq of each step/DNJ eq of the freeze-dried powder of the fermentation broth.

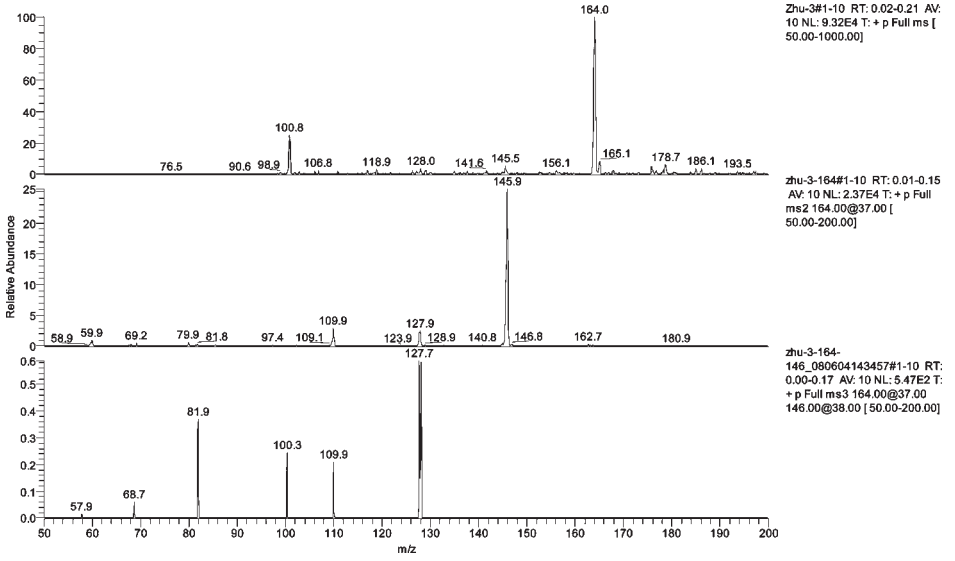

Figure 3. Full scan MS-MS spectrum of the active compound. RT means retention time; AV 10 means the spectrum we see is the average spectrum of 10 times; NL means the maximum signal strength. MS2 was the fraction of the parent ion (*m*/*z* 164), and MS3 was the fraction of the daughter ion (*m*/*z* 146).

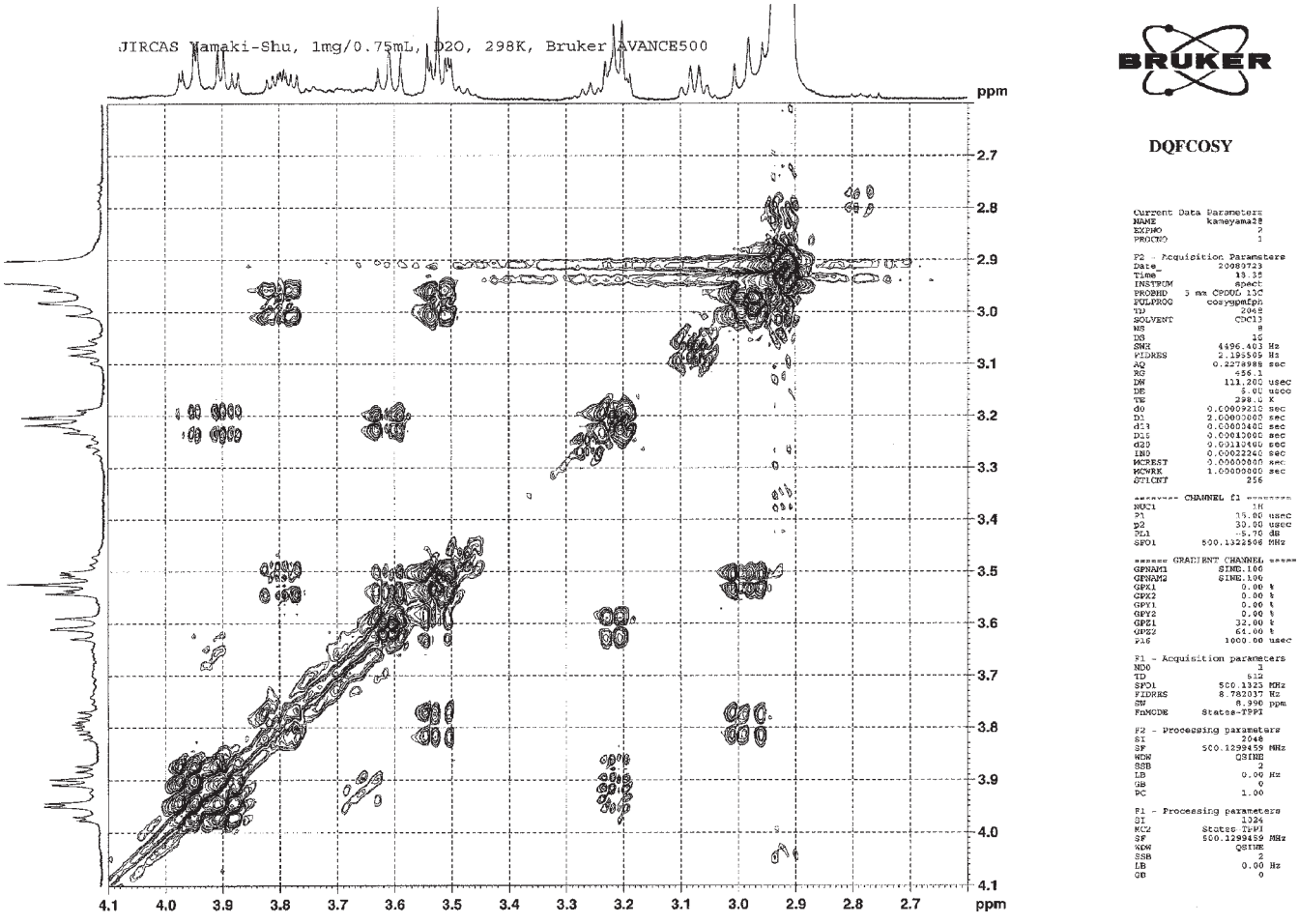

Figure 4. DQFCOSY of the purified content.

nonspecificity (*24*, *25*) while the natural α-glucosidase inhibitors are not easily produced in large scale (*22*, *23*, *26*). Comparatively, production of α-glucosidase inhibitor by *B. subtilis* B2 using okara as the only culture exhibited two characteristics: first, the relatively low cost of okara and its effectiveness in enhancing production of α-glucosidase inhibitor; second, the fast-growing characteristic of *B. subtilis* B2 and its originality from the food source. This result indicated okara could be a potential culture for producing α-glucosidase inhibitor by *B. subtilis* B2.

Purification of the α-Glucosidase Inhibitor. The lyophilized powder of the supernatant and the precipitation after ethanol precipitation were dissolved in 5 mL of distilled water, respectively, to determine their α-glucosidase inhibitory activity. The supernatant contained about 83% of the active activity (**Table 1**)

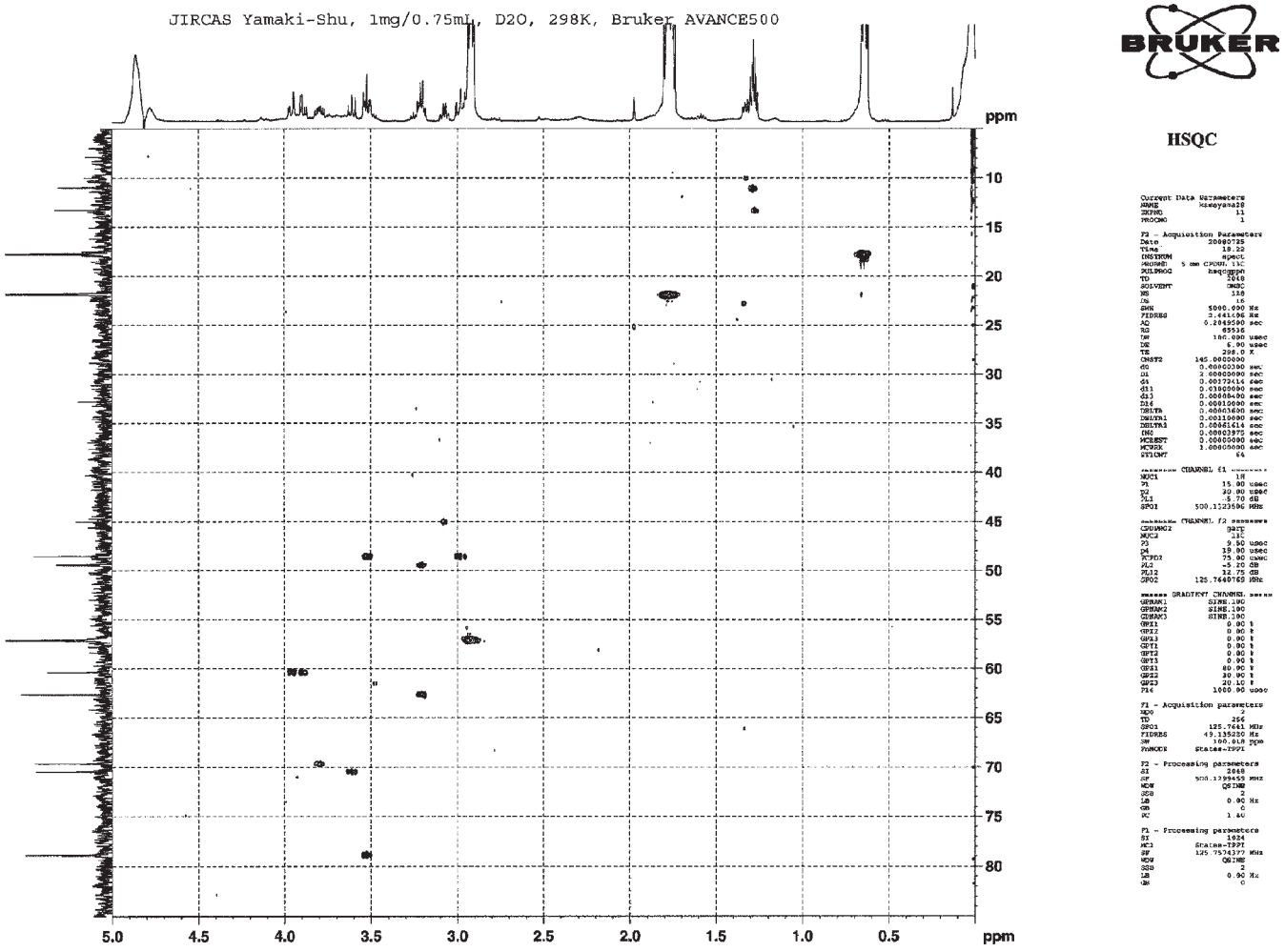

Figure 5. HSQC of the purified content.

Table 2. Chemical Shifts of ^{1}H and ^{13}C (ppm) in NMR Spectrometry

active compound in sample			1-deoxynojimycin (DNJ)		
^{13}C	^{1}H	*J* (Hz)	^{13}C	^{1}H	*J* (Hz)
78.894	3.530t[a]	9.3	79.008 C-3	3.515t	9.9
70.454	3.611t	10	70.650 C-4	3.592t	9.9
69.632	3.796m[b]		69.837 C-2	3.782m	
62.614	3.208		62.642 C-5	3.184m	
60.35	3.955dd[c]	12.8, 3.1	60.547 C-6	3.953dd	12.7, 2.9
	3.895dd	12.7, 5.2		3.878dd	12.6, 5.3
57.089	2.922DSS[d]	IS[e]			
49.403	3.195				
48.533	3.505t	12.4	48.676 C-1	3.508t	12.7
	2.982t	12.1		2.959t	12.0

[a] t means triplet peak. [b] m means multiple peak. [c] dd means double double peak. [d] DSS (2,2-dimethylsilapentane-5-sulfonic acid) is the internal standard in ^{1}H NMR spectrometry. [e] IS means indirect standard.

while the inhibitory activity in the protein and polysaccharide precipitation was very low (data not shown), which indicated that most of the active compound was contained in the supernatant and was not a protein or polysaccharide.

After dialysis using the dialysis membranes with 100 and 1000 Da, the sample was separated into three parts: part I contained the compounds with molecular weight lower than 100 Da; part II contained the compounds with MW from 100 to 1000 Da; and part III contained the compounds with MW higher than 1000 Da. All three parts exhibited α-glucosidase inhibitory activity: part II showed the lowest α-glucosidase IC_{50} value of 13 μg/mL (**Table 1**), while part I and part III showed 250 μg/mL and 64 μg/mL, respectively (data were not shown in the table). Thus, part II was used for the further purification to obtain the α-glucosidase inhibitor.

With active charcoal chromatography, the α-glucosidase inhibitory activity of the eluent began to be detected on the ethanol gradient at a concentration of 10% (calculated based on the gradient curve). In the following 200 min, the active compounds were washed off continuously (data were not shown). The eluents with high activity were concentrated and applied to the CM-sepharose ion exchanger chromatograph. Most of the active compounds were washed off using the formic acid solution at pH 3.7 (data were not shown).

A summary of the purification of the α-glucosidase inhibitor in the fermented okara broth is shown in **Table 1**. After preparative TLC separation, the IC_{50} of the sample decreased to 0.2 μg/mL and the final recovery was 40.3%.

Purity Determination. The comparison of DNJ reference standard and the sample on the HPLC chromatography is shown in **Figure 2**. On the chromatogram (B), except the solvent peak with the retention time of 5.5 min and the unknown minor peak with the retention time of 10 min, the retention time of the main peak was 17.2 min, which was similar to that of the DNJ standard. In the further analyses, MS and NMR were performed to identify the structure of the active compound.

Identification of the Structure of the α-Glucosidase Inhibitor. In the full-scan MS spectrum, a peak at *m*/*z* 164.1 (M^{+} + 1) was shown in **Figure 3**. The fragment ions (daughter ions) of the

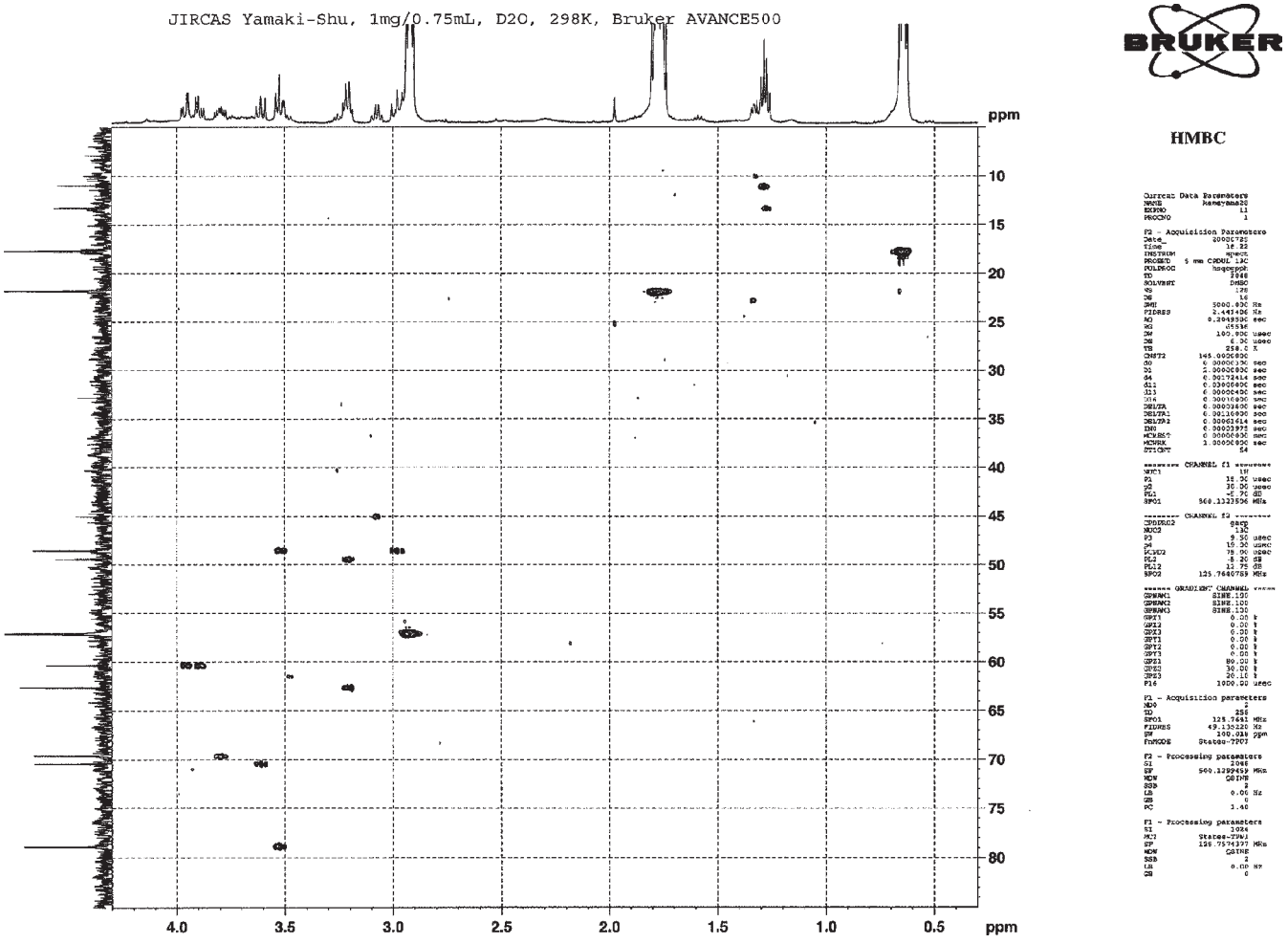

Figure 6. HMBC of the purified content.

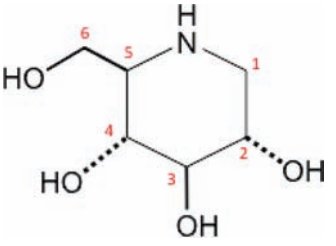

Figure 7. Three-dimensional chemical structure of the purified content. Numbers show the location of carbons.

parent ions (*m*/*z* 164.1) were shown in MS2, and the further fragment ions of the daughter ion (*m*/*z* 145.9) were shown in MS3. By comparison to the MS-MS spectrum of the standard DNJ, the fragment ion information of the MS2 and MS3 of the purified sample was almost the same with the standard DNJ, which indicates that the major compound in the current sample should be DNJ.

The chemical shifts of ^{1}H NMR and ^{13}C NMR are shown in **Table 2** in comparison with those of DNJ standard. These data show that the major content of the purified active sample is DNJ. From the spectrum of the DQF-COSY (**Figure 4**), HSQC (**Figure 5**), and HMBC (**Figure 6**), the three-dimensional structure of the content has been determined (**Figure 7**).

As **Table 1** showed, the concentration of DNJ in the freeze-dried fermentation broth is about 0.74 mg/g in the current research. The concentration of the DNJ is similar to the average concentration of DNJ in mulberry leaves collected from whole branches of the different cultivar (*10*), while it is lower than the DNJ concentration of 0.75 g/L produced by *B. subtilis* S10, which was isolated from soil (*27*). Actually, the amount of DNJ usually highly depends on the carbon source (*28*) and might be improved by optimization of the culture media by mutagenic treatments (ultravilolet irradiation or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine treatment) (*29*). Although the current concentration of DNJ produced by *B.subtilis* B2 is not as much as that produced by *B. subtilis* S10, improvement of the DNJ concentration will be performed in our future work.

Several reports have investigated the efficacious dose of DNJ *in vivo* (*10*, *27*). The food-grade mulberry powder enriched with DNJ (15 mg/g) could significantly suppress the elevation of postprandial blood glucose in humans. In the current study, the concentration of DNJ purified by activated charcoal chromatography reached 12.5 mg/g, which might be the efficacious concentration for maintaining the blood glucose level. In our future study, the bioavailability of the DNJ-derived products fermented by *B. subtilis* B2 in animals will be investigated.

Although a few researchers have reported that DNJ was synthesized by *Bacillus* species isolated from soil (*7*, *11*), we first reported the Bacillus capable of producing DNJ isolated from food sources. Therefore, *B. subtilis* B2 might be used as the start strain for production of the food-derived DNJ products as functional foods for diabetic patients.

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