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Original Research Article

Improvement of 2-O-α-D-Glucopyranosyl-L-Ascorbic Acid Biosynthesis Using Ultrasonic Radiation

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Abstract

Purpose: *To improve 2-O-α-D-glucopyranosyl-L-ascorbic acid (AA-2G) production using ultrasonic radiation (UR) treatment.*
Methods: *The production of AA-2G using UR or ultrasonic radiation with shaking (URS) at 150 rpm, at varying power (100 − 500 W), temperature (30 – 65 °C), pH 4.0 −9.0, and time (2−24 h) was compared with that produced in a shaker water bath (SWB) in a reaction catalyzed by cyclodextrin glucanotransferase (CGTase) from Bacillus sp. SK13.002. The effect of URS on CGTase activity was also measured.*
Results: *Maximum AA-2G production using UR at a power of 400 W, temperature of 37 °C, and pH 8.0 for 18 h was 5.69 ± 0.2 g/L, while URS at 500 W/150 rpm and 37 °C for 14 h yielded 7.05 ± 0.21 g/L of AA-2G. URS at 500 W/150 rpm, 55 °C, and pH 8.0 for 6 h yielded 6.6 ± 0.25 g/L of AA-2G. URS at 37 and 55 °C significantly increased CGTase activity. AA-2G yield using UR (400 W) was decreased by 9.7 % compared to that produced by SWB. However, the AA-2G yield using USS (500 W/150 rpm) at 37 and 55 °C increased by 11.9 and 4.8 %, respectively, with a reduction in process time of 41.7 and 75 %, respectively, compared to that previously produced by SWB.*
Conclusion: *These results indicate that UR combined with shaking improves AA-2G production.*

Keywords: *2-O-α-D-glucopyranosyl-L-ascorbic acid, Ultrasonic radiation, Transglycosylation, Bacillus sp. SK13.002*

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INTRODUCTION

The compound 2-O-α-D-glucopyranosyl-L-ascorbic acid (AA-2G) is a glycoside form of L-ascorbic acid (AA). It is enzymatically synthesized via transglycosylation by mammalian α-glucosidase. Unlike AA, AA-2G is stable in the presence of enhanced oxidative degradation by heat, light, Cu^{2+} ion, or ascorbate oxidase, and it has no reducing activity toward radicals. AA-2G is available *in vitro* and *in vivo* as AA; therefore, it is used in cosmetics, medicine, and the food industry [1]. Increasing production of AA-2G using highly soluble and low cost glycosyl donors such as maltose and maltodextrin was recently reported [2,3], but the yield was still low compared to other substrates. Maximum production of AA-2G has also been reported, using different substrates and enzymes in reactions lasting 24−48 h [4].

Ultrasonic irradiation (UR) has been shown to increase the yield in various processes that involve both enzymatic and non-enzymatic reactions. However, UR decreased the times of the chemical reactions, while enzyme-catalyzed reactions were accelerated by UR [5]. Although enzymatic transglycosylation reactions have been accelerated by microwave radiation [6], to the best of our knowledge, there have been no published reports on the effects of UR on transglycosylation reaction using AA for AA-2G production. Recently we used maltodextrin as a glycosyl donor for production of AA-2G via a transglycosylation reaction catalyzed by cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) from *Bacillus* sp. SK13.002 [7]. The aim of the present study was therefore to characterize the production of AA-2G under UR by using maltodextrin as a substrate.

EXPERIMENTAL

Materials

The AA-2G standard was obtained from Hayashibara Biochemical Laboratories (Okayama, Japan), while AA and glucoamylase were obtained from Sigma-Aldrich (Shanghai, China). Maltodextrin (DE 10-15) was purchased from the Grain Processing Corporation (Muscatine, IA, USA). All other analytical grade chemicals were purchased from Sinopharm Chemical Reagent Co Ltd. (Shanghai, China).

Evaluation of hydrolytic activity of CGTase

The hydrolytic activity of CGTase was measured by assaying the hydrolysis effect on starch, as previously described [6]. Briefly, based on the decrease of blue color intensity of the amylose-iodine complex formed after reacting CGTase with 0.3 % (w/v) soluble starch in 200 mM phosphate buffer (pH 8.0) at 65 °C and measured at 660 nm. One unit of CGTase activity was defined as the amount of enzyme that catalyzed a 10 % decrease of absorbance per min under the assay conditions.

Determination of cyclization activity of CGTase

The cyclization activity of CGTase was measured by the phenolphthalein method as previously described [8]. Briefly, a reaction mixture containing 1 mL of 0.04 g starch in 0.1 M phosphate buffer (pH 6.0) and 0.1 mL enzyme solution was used. The mixture was incubated at 60 °C for 10 min in a water bath. The reaction was stopped by adding 3.5 mL of 0.03 M NaOH solution, 0.5 mL of 0.02 % (w/v) phenolphthalein in 0.005 M Na_2CO_3 was added to the reaction mixture. After 15 min, the decrease in color intensity was measured at 550 nm. One unit of CGTase activity was defined as the amount of enzyme that forms 1 mmol β-cyclodextrin per min under the assay conditions.

Partial purification of CGTase

Bacillus sp. SK13.002 originally was isolated from a soil sample in our laboratory and screened by phenolphthalein – methyl orange method, it was found to produce CGTase [9]. The crude enzyme was obtained after fermentation for 4 days and centrifugation at 10,000 rpm and 4 °C for 15 minutes. The crude preparation was concentrated, precipitated, dialyzed, and freeze-dried as previously described [6]. The resulting CGTase powder was used in all subsequent experiments.

Ultrasonic equipment

An experimental ultrasonic cleaner (model KQ500DE; Ultrasonic Equipment Co., Ltd., Kunshan, China) was used in this study. The internal dimension of the cleaning size (mm) was 300 × 300 × 150, with a capacity of 22.5 L. The ultrasonic cleaner could deliver a maximum power of 500 W at 40 kHz with adjustable time, power, and temperature. The temperature was kept constant by a continuous supply of cold tap water and drainage of hot water to prevent a rise in the temperature due to the UR. Ultrasonic radiation with shaking (URS) was constructed by using an arm fixed in a shaking water bath (SWB) (model HZ-8812S-B; Hualida Laboratory Equipment Company, Wenzhou, China) that extended inside the ultrasonic cleaner with a sample holding device.

Biosynthesis of AA-2G

The reaction was carried out using the following formula:

AA + maltodextrin (CGTase) = AA-2G + AA-2Gn (Glucoamylase) = AA-2G + glucose

where AA-2Gn = AA-2-oligosaccharides (n is number of glucose units ranging from 2 to 6).

The reaction mixture consisted of maltodextrin (0.016 g/mL), AA (0.016 g/mL), and 400 U of CGTase in 0.1 M sodium phosphate buffer (pH 8), which was initially incubated at 37 °C for 2 h. Glucoamylase was added to the reaction mixture at pH 5.5 and incubated at 65 °C for an additional 6 h.

Analysis of the reaction mixture for AA-2G production was carried out using an HPLC system consisting of an Agilent Technologies 1200 Series instrument (Alpharetta, GA, USA) as we previously described [7].

Biosynthesis of AA2G using UR

The AA-2G production was first carried out for 2 h using a sonication power of 200 − 500 W, pH 4.0−9.0, temperature of 30 – 65 °C, and a duration of 2 - 24 h. The influence of URS on AA-2G production was studied for 2 h using a sonication power of 200−500 W, pH 4.0 − 9.0, temperature of 30 – 65 °C, different substrate ratios and duration of 2 -18 h.

Statistical analysis

All experiments were performed in triplicate and the mean (± standard deviation) recorded. Analysis of variance (ANOVA) was carried out and the results further evaluated by Duncan's multiple range tests. Statistical software SPSS 17.0 (SPSS Inc, Chicago, IL, USA) was used for the analyses, and $p < 0.05$ was considered statistically significant.

RESULTS

Effect of UR on biosynthesis of AA-2G

Biosynthesis of AA-2G using UR at 37 °C was compared with that obtained by conventional SWB as a control. Maximum production of AA-2G (2.13 ± 0.08 g/L) in 2 h was obtained using an ultrasonic power of 400 W (Figure 1A). This yield represented a significant increase ($p < 0.05$) of 82.05 % compared with that produced by the control (1.17 ± 0.07 g/L). Reaction parameters such as temperature, pH, and duration of time were optimized, resulting in maximum AA-2G yields (5.69 ± 0.2 g/L) at 37 °C and pH 8.0 after 18 h (Figure 1).

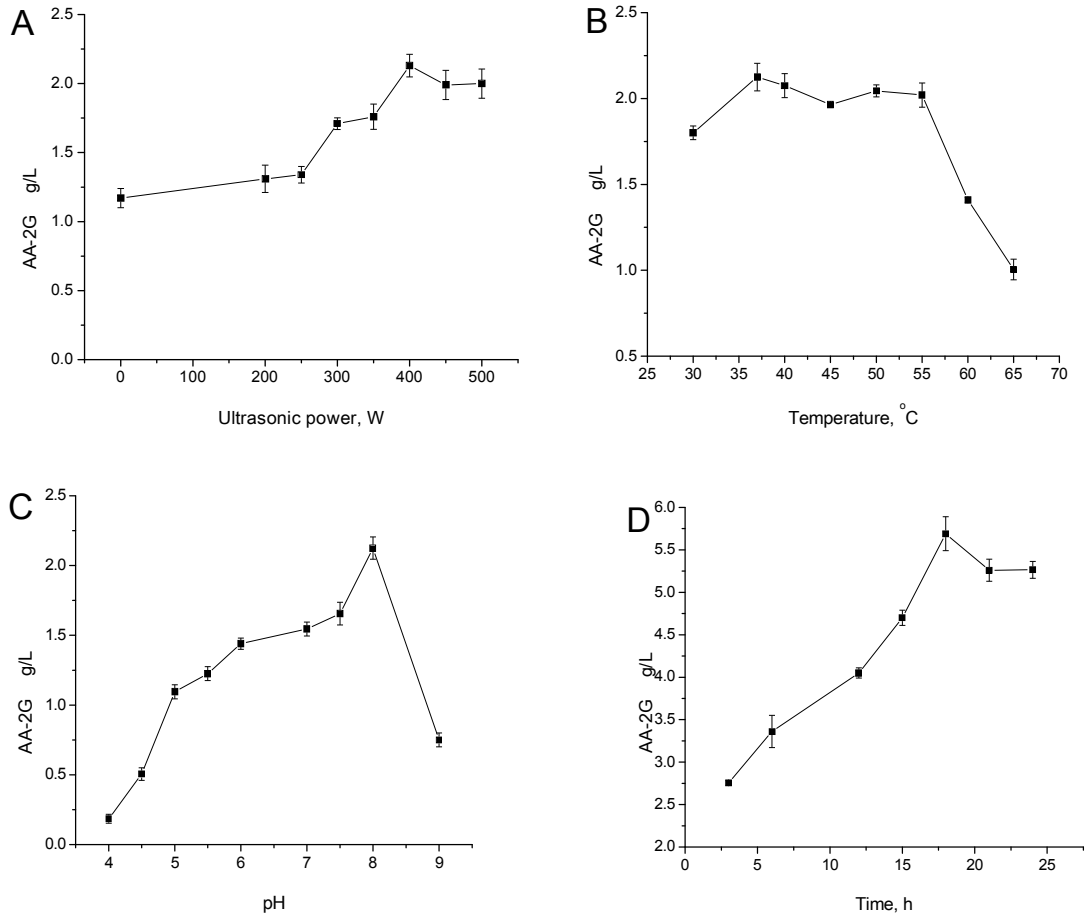

Figure 1: Effect of ultrasonic radiation (UR) on 2-O-α-D-glucopyranosyl-L-ascorbic acid (AA-2G) production. (A) Effects of different power at 37°C for 2 hours, (B) the effects of different temperatures at 400 W for 2 hours, (C) the effects of different pH at 400 W and 37°C for 2 hours, and (D) the effects of different duration times at pH 8.0, 400 W, and 37°C

Effect of URS on biosynthesis of AA-2G

Transglycosylation of AA during production of AA-2G under UR with shaking at 150 rpm (URS) was carried out with the same reaction mixture as described in the previous section. Maximum production of AA-2G (2.78 ± 0.10 g/L) was obtained at 500 W (Figure 2A). This yield represented a significant increase ($p < 0.05$) of 137.6 % compared with that produced by the control (1.17 ± 0.07 g/L). The influences of reaction time at pH 8.0 and 37 °C were also assessed, showing that the maximum production of AA-2G using URS for 14 h was 7.05 ± 0.21 g/L (Figure 2B).

Reaction parameters such as temperature, pH, substrate ratios, and duration for AA-2G production using URS were optimized. URS induced a shift of the optimum temperature from 37 to 55 °C for 2 h, which was accompanied by a significant increase ($p < 0.05$) in AA-2G production from 2.78 ± 0.10 g/L to 3.31 ± 0.08 g/L (Figure 3A). At 55 °C, pH 8.0, and a 1:1 ratio (AA: maltodextrin), the reaction produced AA-2G yield of 6.6 g/L in 6 h.

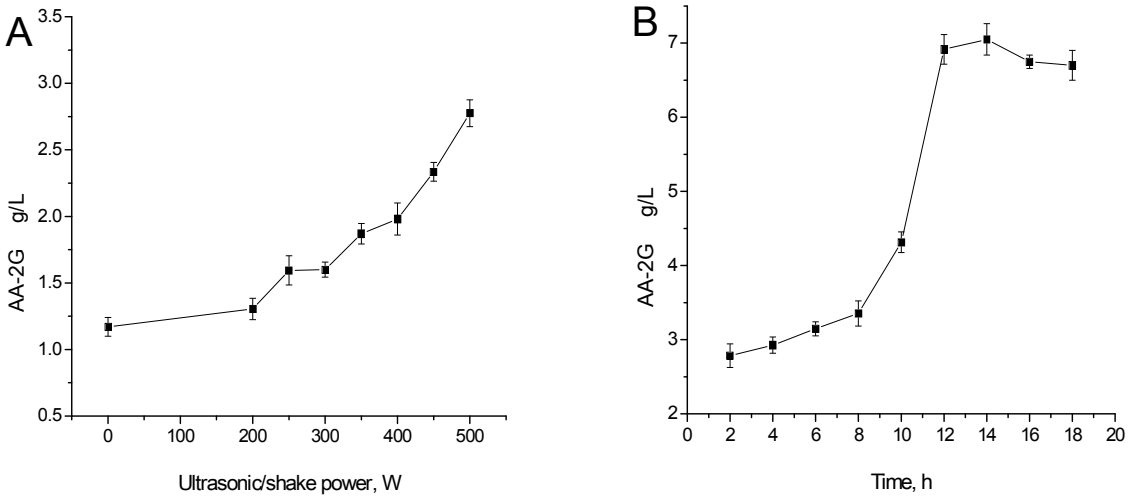

Figure 2: Effect of ultrasonic radiation with shake (URS) on AA-2G production at 37°C and 150 rpm. (A) The effects of different power for 2 hours and (B) duration times at 500 W

Effect of URS on CGTase activity

The influence of URS on hydrolytic and cyclization activities of CGTase from Bacillus sp. SK13.002 was determined by assaying the activities directly with URS (500 W/150 rpm). In addition, both activities were measured after USS (500 W/150 rpm) pretreatment at 37 and 55 °C for 2 h. Table 1 shows a significant increase ($p < 0.05$) in hydrolytic and cyclization activities either directly measured with USS or after pretreatment for 2 h, when compared to the activities of the control, directly measured with conventional SWB.

DISCUSSION

There have been recent improvements of AA-2G production by using maltodextrin as a highly soluble and low cost substrate [3,10,11]. Previously, we obtained a maximum yield of AA-2G (6.3 g/L) using the following conditions: 24 h reaction time, pH 8, 1:1 AA to maltodextrin ratio, and a temperature of 37 °C [7]. In the present study, we used UR and URS to assess the improvement of AA-2G production and to compare the present and previous results. The present results showed that both UR either without shaking or with shaking and short time treatment (2 hours) significantly increased AA-2G production by 82.05 % and 137.6 %, respectively, compared to the control group. In addition, CGTase hydrolytic and cyclization activities, either measured directly under URS or after pretreatment by URS, were significantly increased. These results indicated that AA-2G production was improved due to activation of CGTase. Generally, UR in liquids induced heating and cavitation effects, and improved mass transfer [12]. As a result of this mass transfer, enzyme activity was enhanced due to the movement of reactants to the active site of the enzyme [13]. The hydrolysis rate for 2 h under shaking has been found to increase by 64 % as a result of UR compared to the control samples, which was attributed to less enzyme

agglomeration and easier dispersion induced by UR [14].

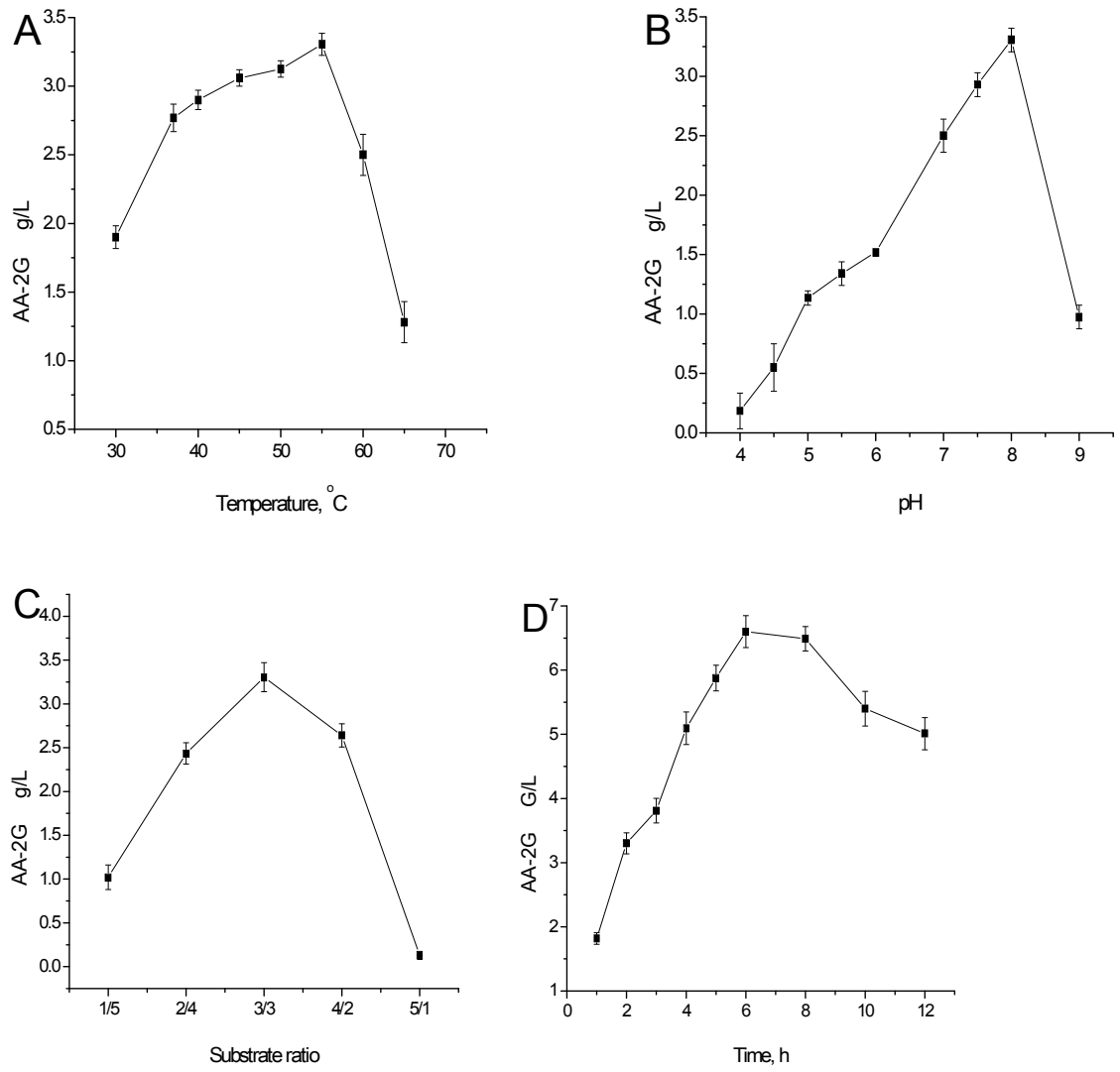

Figure 3: Effect with URS at 500 W/150 rpm of temperature (A), pH (B), substrate ratio (C), and duration of time (D)

Table 1: Effect of URS (500 W/150 rpm) treatment on hydrolysis and cyclization activities of CGTase

Process conditions	Type of CGTase	Hydrolytic activity (U/mg)*	Cyclization activity (U/mg)*
Direct	Control	355.00 ± 4.33[a]	54.03 ± 1.09[a]
Direct	URS	366.67 ± 1.11[b]	55.73 ± 0.70[b]
Pretreatment for 2 h	URS at 37°C	363.0 5± 3.19[b]	57.56 ± 0.71[c]
Pretreatment for 2 h	URS at 55°C	369.29 ± 4.62[b]	61.56 ± 0.43[d]

Results represent the mean ± standard deviation (SD, n = 3); values with different superscript in the same column significantly are different ($p < 0.05$) using Duncan's least significant test

Treatment by UR produced an AA-2G yield of 5.69 ± 0.2 g/L which was less than that previously produced (6.3 g/L) using SWB. However, URS either at 37 °C or 55 °C increased the AA-2G yield by 11.9 % and 4.8 %, respectively, and reduced the process times by 41.7 % and 75 %, respectively, compared to our previous findings. These results indicated that shaking had a synergistic effect with UR, resulting in an improved AA-2G yield and reduced process time. This improvement could be due to acceleration of enzyme and substrate

collisions, acceleration of substrate and product diffusion to and from the enzyme active site, and mild UR radiation-induced enzyme activation [15]. Previously, UR was found to increase reaction rates and yields of sugar ester biosynthesis, which were attributed to acceleration of the collision probability of enzyme and substrate [5].

CONCLUSION

The results of the present study demonstrated that UR could be used to carry out the transglycosylation reaction for production of AA-2G. Although the yield of AA-2G using UR was less than that by SWB, the yield and reaction time using URS significantly were improved compared to that to that previously produced using SWB. These finding indicated that shaking has synergistic effect with UR for improving AA-2G production.

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REFERENCES

1. *Han R, Liu L, Li J, Du G, Chen J. Functions, applications and production of 2-O-D-glucopyranosyl-l-ascorbic acid. Appl Microbiol Biotechnol 2012; 95: 313-320.*
2. *Liu L, Han R, Shin HD, Li J, Du G, Chen J. Biosynthesis of 2-O-D-glucopyranosyl-L-ascorbic acid from maltose by an engineered cyclodextrin glycosyltransferase from Paenibacillus macerans. Carbohydr Res 2013; 382: 101–107.*
3. *Liu L, Xu Q, Han R, Shin HD, Chen RR, Li J, Du G, Chen J. Improving maltodextrin specificity for enzymatic synthesis of 2-O-D-glucopyranosyl-L-ascorbic acid by site-saturation engineering of subsite-3 in cyclodextrin glycosyltransferase from Paenibacillus macerans. J Biotechnol 2013; 166: 198-205.*
4. *Markosyan AA, Abelyan LA, Adamyan MO, Akopyan ZI, Abelyan VA. Transglycosylation of L-Ascorbic Acid. Appl Biochem Biotechnol 2007; 43: 36–40.*
5. *Xiao YM, Wu Q, Cai Y, Lin XF. Ultrasound-accelerated enzymatic synthesis of sugar esters in nonaqueous solvents. Carbohydr Res 2005; 340: 2097-2103.*
6. *Sun T, Jiang B, Pan B. Microwave accelerated transglycosylation of rutin by cyclodextrin glucanotransferase from Bacillus sp. SK13. 002. Int J Mol Sci 2011; 12: 3786-3796.*
7. *Eibaid A, Miao M, Bashari M, Musa A, Jiang B. Biosynthesis of 2-α-D-glucopyranosyl-L-ascorbic acid from Maltodextrin Catalyzed by Cyclodextrin Glucanotransferase from Bacillus sp. SK13. 002. Journal of Academia and Industrial Research 2014; 2: 627- 630.*
8. *Kaneko T, Kato T, Nakamura N, Horikoshi K. Spectrophotometric determination of cyclization activity of β-cyclodextrin-forming cyclomaltodextrin glucanotransferase. J Jpn Soc Starch Sci 1987; 34: 45-48.*
9. *Park C, Park k, Kim S. A Rapid Screening Method for Alkaline β-Cyclodextrin Glucanotransferase using Phenolphthalein-Methyl Orange-containing-solid Medium. Agric Biol Chem 1989; 53:1167-1169.*
10. *Han R, Liu L, Shin HD, Chen RR, Du G, Chen J. Site-saturation engineering of lysine 47 in cyclodextrin glycosyltransferase from Paenibacillus macerans to enhance substrate specificity towards maltodextrin for enzymatic synthesis of 2-O-D-glucopyranosyl-L-ascorbic acid (AA-2G). Appl Microbiol Biotechnol 2013; 97: 5851-5860.*
11. *Han R, Liu L, Shin HD, Chen RR, Li J, Du G, Chen J. Systems Engineering of Tyrosine 195, Tyrosine 260, and Glutamine 265 in Cyclodextrin Glycosyltransferase from Paenibacillus macerans to Enhance Maltodextrin Specificity for 2-O-D-Glucopyranosyl-L-ascorbic Acid Synthesis. Appl Environ Microbiol 2013; 79: 672-677.*
12. *Basto C, Silva CJ, Gübitz G, Cavaco-Paulo A. Stability and decolourization ability of Trametes villosa laccase in liquid ultrasonic fields. Ultrason Sonochem 2007; 14: 355-362.*
13. *Wang J, Cao Y, Sun B, Wang C, Mo Y. Effect of ultrasound on the activity of alliinase from fresh garlic. Ultrason Sonochem 2011; 18: 534–540.*
14. *Liu Y, Jin Q, Shan L, Liu Y, Shen W, Wang X. The effect of ultrasound on lipase-catalyzed hydrolysis of soy oil in solvent-free system. Ultrason Sonochem 2008; 15: 402-407.*
15. *Zhang DN, Guo XY, Yang QH, Chen ZG, Tao LJ. An efficient enzymatic modification of cordycepin in ionic liquids under ultrasonic irradiation. Ultrason Sonochem 2014; 21: 1682-1687.*