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Establishment of a rapid and effective plate chromogenic assay for screening of Aspergillus species with high β-fructofuranosidase activity for fructooligosaccharides production

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ABSTRACT

Fructooligosaccharides (FOS) are commonly regarded as prebiotics and used as components of functional foods. Currently, the industrial sucrose-to-FOS biotransformation is mainly carried out using the microbial-derived β-fructofuranosidases with transglycosylation activity as catalysts. Evaluation of the ability of a microorganism to produce β-fructofuranosidase is commonly conducted by measuring enzyme activity. However, the traditional method requires several steps to identify strains with high β-fructofuranosidase activity, which is not suitable for high-throughput screening. To facilitate screening of a large number of microbial cultures, this study developed a plate chromogenic assay method based on the glucose oxidase (GOD) - peroxidase (POD) bienzymatic system for screening of β-fructofuranosidase-producing fungal strains and predicting their potential to produce FOS. This method used the amount of glucose released from sucrose as indicator to form clear pink halos around the microbial colonies with β-fructofuranosidase activity. Cultivation conditions for the plate assay were optimized as cultivation time 5 h and spore inoculum concentration 10^8/ml. Moreover, the method was applied to screening of an Aspergillus niger ATCC 20611 mutant library. The mutant A11 displaying the largest pink halo was screened out and its β-fructofuranosidase activity was determined to be 1.65 fold than that of the parental strain. Thin layer chromatography (TLC) assay further indicated that A11 with the largest halo possessed the highest FOS synthesis ability. These results demonstrated the potential of this plate chromogyenic assay method in the rapid and effective identification of excellent FOS producers from a large number of strain samples.

1. Introduction

With the rising living standards and growing health consciousness, people hope that foods can not only provide energy for the body, but also regulate body functions and improve health. Prebiotics used as functional food ingredients can have a positive influence on health, and consequently have great demand in global food markets (Ashwini et al., 2019; Dominguez et al., 2014). Among them, fructooligosaccharides (FOS) have gained particular attention because of their important biological properties and excellent physiological functions (Dominguez et al., 2014; Maiorano et al., 2008). FOS are a generic term for a series of homologous fructose oligomers and mainly consist of kestose (GF2), nystose (GF3) and 1^F-β-fructofuranosyl nystose (GF4) (Maiorano et al., 2008). FOS can be found in some natural foods and crops (Ashwini et al., 2019), but these plant materials are subject to seasonal restrictions. In addition, it is difficult to extract FOS from these materials (Dominguez et al., 2006). Commercially, food-grade FOS can be produced by binding 1–3 fructosyl units to the fructosyl unit of sucrose using β-fructofuranosidase (FFase, EC 3.2.1.26) or fructosyltransferase (FTase, EC 2.4.1.9) as catalyst (Sangeetha et al., 2005a). These FOS-producing enzymes can be mostly derived from fungi, such as Aspergillus niger (Hidaka et al., 1988), Aspergillus japonicus (Chien et al., 2001), Aureobasidium pullulans (Lateef et al., 2007), and Penicillium expansum (Prata et al., 2010). Among these fungi, Aspergillus species have been considered as excellent β-fructofuranosidase producers (Maiorano et al., 2008). For example, Aspergillus niger ATCC 20611 has been considered as one of the most suitable strains used in commercial FOS production (Hidaka et al., 1988). However, the cost of β-fructofuranosidase production is still a limiting factor in the FOS industry to meet the growing market needs (Nascimento et al., 2016).

Efforts on optimization of nutritional and cultivation parameters of fermentation processes to increase the β-fructofuranosidase yield have

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been made (Sangeetha et al., 2005b; Mutanda et al., 2014). Neverthless, the fermentation optimization possesses an upper limit to the degree of enzyme production that can be increased (Zhang et al., 2017). It is known that screening of new FOS-producing strains with higher β-fructofuranosidase yields could offer a great opportunity for cost reduction (Zhang et al., 2017; Xie et al., 2017). However, rapid screening of the β-fructofuranosidase-producing strains is still difficult due to the lack of suitable high-throughput screening methods. Current methods for evaluating the ability of a strain to produce β-fructofuranosidase mainly depend on measuring enzyme activity, but determination of β-fructofuranosidase activity is complex and time-consuming (Nascimento et al., 2016; Fernandez et al., 2007; Wang and Zhou, 2006). To facilitate screening a large number of microbial cultures, it is necessary to develop a simpler assay method to screen more samples in shorter time. Dominguez et al. (2006) had reported a plate chromogenic test for screening of fungi with transfructosylation activity by detecting both glucose and fructose released from the sucrose. This method needed not only the glucose oxidase (GOD) - peroxidase (POD) system to determine glucose, but also the fructose dehydrogenase to determine fructose. However, the fructose dehydrogenase was difficult to be heterologously expressed, and thus it was commercially expensive (Kawai et al., 2013).

In fact, most microbial-derived β-fructofuranosidases naturally possess transfructosylation activity at high sucrose substrate concentration, which can transfer the fructosyl group to synthesize FOS and simultaneously release glucose (Maiorano et al., 2008). Thus, the concentration of glucose could be potentially used for evaluating the β-fructofuranosidase activity and indirectly reflect the ability of the strains to synthesize FOS. In principle, in this GOD - POD bienzymatic system, GOD oxidizes glucose to produce hydrogen peroxide, and then POD decomposes it into water and oxygen, simultaneously makes the substrates, 4-aminoantipyrine (4-AAP) and phenol, to be dehydrocondensed to form pink quinone compounds (Dominguez et al., 2006). Therefore, a plate chromogenic assay based solely on the GOD - POD bienzymatic system was developed in this study to detect the β-fructofuranosidase activities produced by the fungal strains. The method was successfully applied to screening of the high-yield β-fructofuranosidase-producing strains with high transglycosylation activity from a random mutagenesis library of *A. niger* ATCC 20611.

2. Materials and methods

2.1. Strains and cultivation media

Aspergillus niger ATCC 20611, which has high β-fructofuranosidase activity, was used as the parental strain. The mutant strains were obtained by UV mutagenesis. Czapek Dox (CD) agar medium consisted of sucrose 30 g/L, $NaNO_3$ 3 g/L, K_2HPO_4 1 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, KCl 0.5 g/L, $FeSO_4$ 0.01 g/L, $CuSO_4$ 0.01 g/L and agar 20 g/L. Potato dextrose agar (PDA) medium contained peeled potato extract 200 g/L, D-glucose 20 g/L and agar 20 g/L. Fermentation medium (FM) contained sucrose 50 g/L, yeast extract 15 g/L, KCl 0.5 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, K_2HPO_4 5 g/L and $NaNO_3$ 2 g/L at pH 5.0.

2.2. Development of the plate chromogenic assay

The plate chromogenic assay was conducted as follows: 1 μL of spores was spotted onto CD agar plates and incubated at 30 °C. After incubation, the colonies were overlaid with the GOD - POD bienzymatic system (a biochemical reaction system, composed of 5 U/mL glucose oxidase (GOD), 0.5 U/mL horseradish peroxidase (POD), 0.1 mg/mL 4-aminoantipyrine, 1 mg/mL phenol and 7 mg/mL agar powder) and reacted at 40 °C for 10 min. The results were obtained by measuring the formation of pink halos around the colonies.

2.3. Optimization of the plate chromogenic assay

2.3.1. Cultivation time

1 μL aliquots of each fungal spore suspension (10^7/mL) were spotted onto CD agar plates and cultivated at 30 °C for 2 h, 5 h, 10 h, 24 h and 36 h, then the GOD-POD bienzymatic system was poured into the plates. After the chromogenic reactions were completed, the halo diameters were measured. Three biological replicates were performed for each strain and the data were shown as the mean and SD of three replicate cultures.

2.3.2. Spore inoculum concentration

1 μL of spores at the concentration of 10^6/mL, 10^7/mL, 10^8/mL and 10^9/mL was spotted onto CD agar plates, respectively. The optimal cultivation time determined in 2.3.1 was used as the incubation time for subsequent experiments. After the chromogenic reactions finished, the diameters of the pink halos were measured to determine the optimal spore inoculum concentration. Three biological replicates were performed for each strain and the data were shown as the mean and SD of three replicate cultures.

2.4. Random mutagenesis

2 ml of ATCC 20611 spores (10^6/mL) was poured into plates, then the plates were placed at a distance of 30 cm from UV lamp (20 W G15 T8, Philips Ltd.) for 90s. The mutagenized spores were diluted to 10^3/mL and spread onto MM plates. After incubated at 30 °C for 3d, the mycelia of mutant strains were picked out and placed on the PDA plates for conidia production.

2.5. Evaluation of the ability of mutant strains to produce β-fructofuranosidase by plate chromogenic assay

The spores of the randomly selected mutant strains and the parental strain ATCC 20611 were spotted onto CD plates. After incubation at 30 °C for the optimal cultivation time, the GOD - POD bienzymatic system was added to the plates. When the chromogenic reactions were completed, the diameters of the pink halos formed by the mutant strains and ATCC 20611 were compared. Three biological replicates were performed for each strain and the data were shown as the mean and SD of three replicate cultures. Statistical analysis was performed by using Student's *t*-test. Significance different at $P < .05$ or $P < .01$ was indicated in figure by one or two asterisks, respectively.

2.6. Determination of β-fructofuranosidase activity

The spores (10^8/mL) of the mutant strains and ATCC 20611 were transferred to a 500-mL shaken flask containing 200 mL of FM. After incubation at 30 °C for 48 h, the cultures were centrifuged and the mycelia cells were collected for β-fructofuranosidase activity assay and FOS synthesis.

The β-fructofuranosidase activity assay was performed by adding the collected mycelia cells to a reaction mixture containing 25% sucrose (*w*/w) and 0.1 M citrate buffer (pH 5.0), then the mixed system was reacted at 50 °C for 2 h. Assay was terminated by heating the mixture in the boiling water for 10 min. The β-fructofuranosidase activity was determined by the 3, 5-dinitrosalicylic acid (DNS) method as described previously (Ghazi et al., 2007). One unit (U) of β-fructofuranosidase represented the amount of enzyme required to produce 1 μmol of glucose per minute. Three biological replicates were performed for each strain and the results were shown as the mean and SD of three replicate cultures. Statistical analysis was performed by using Student's *t*-test. Significance at $P < .01$ was indicated in figure by two asterisks.

2.7. Transglycosylation reaction

The collected mycelia cells were added to 50% (w/w) sucrose solution and incubated at 50 °C for 5 h to perform the transglycosylation reaction and synthesize FOS. The amount of enzyme used was per gram of sucrose corresponding to 6 U β-fructofuranosidase. The reaction was stopped by boiling.

2.8. Thin layer chromatography (TLC) assay

The transglycosylation products were centrifuged at 10,000 rpm for 10 min. Then the sugar composition of the supernatants was identified by thin layer chromatography (TLC). In detail, the supernatants were diluted 20 times, 1 μL of the diluents was spotted 10 mm from the bottom of the silica gel 60 F254 plate (Millipore, Germany). And then the plate was developed using the reagent, which contained butanol, isopropanol, acetic acid and water in volume at proportions of 7:5:2:4, respectively. The sugars were visualized after using a spray of diphenylamin-aniline-phosphoric acid (DPA) reagent and reacting at 110 °C for 10 min.

3. Results

3.1. Optimization of cultivation conditions for the plate chromogenic assay

To determine the appropriate culture conditions for the plate chromogenic assay, cultivation time and spore inoculum concentration were optimized. Fig. 1. shows the effect of the cultivation time on the chromogenic reaction with *A. niger* ATCC 20611 used as the test fungal strain. It was found that the pink halo around the fungal colony could be seen when cultivation for 2 h and the areas of the halos increased as the cultivation time extended (Fig. 1A). After incubation for 10 h, the diameter of the pink halo had exceeded 3.0 cm, which was more than one-third of that of the plate (Fig. 1B). Considering that multiple fungal colonies could be distinguished in one plate, 5 h was used as the optimal cultivation time for subsequent assays.

In order to find the most appropriate spore inoculum concentration,

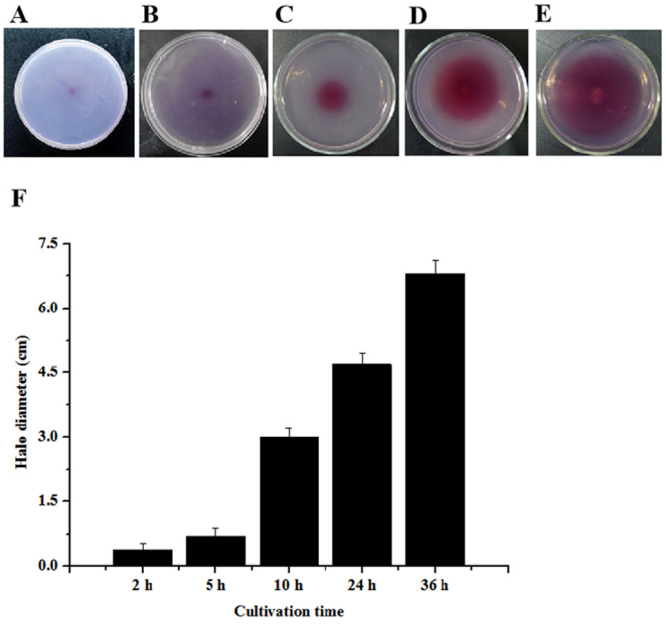

Fig. 1. Effects of the cultivation time on the GOD-POD bienzymatic system. Aspergillus niger ATCC 20611 was spotted onto CD medium for 2 h (A), 5 h (B), 10 h (C), 24 h (D), 36 h (E) respectively. (F) The diameters of the halos formed around the colonies. Error bars represent standard deviation of three replicate cultures.

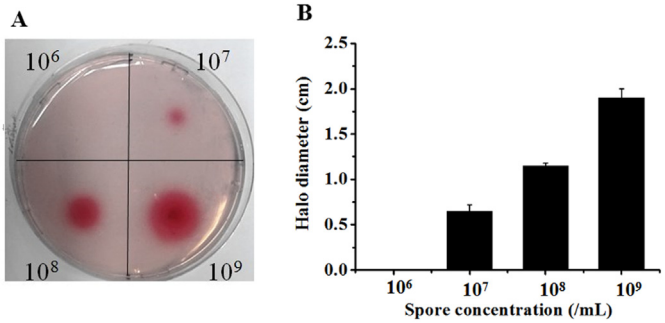

Fig. 2. Effects of the spore inoculum concentration on the GOD-POD bienzymatic system. (A) Different concentrations of Aspergillus niger ATCC 20611 spores were spotted on the same CD plate for 5 h, then the amount of glucose released was detected by the GOD-POD bienzymatic system. (B) The diameters of the halos formed around the colonies. Error bars represent standard deviation of three replicate cultures.

1 μL of ATCC 20611 spores at concentration of 10^6/mL, 10^7/mL, 10^8/mL and 10^9/mL was spotted onto CD plates and inoculated for 5 h, then the glucose released from sucrose was detected by the GOD-POD bienzymatic system. As shown in Fig. 2, the pink halo could not appear at concentration of 10^6/mL, and became visible at concentration of 10^7/mL. Similar to the effect of the cultivation time on the chromogenic reaction, the diameters of the halos expanded with the concentration of spore suspension from 10^7/mL to 10^9/mL (Fig. 2A). But when the inoculum concentration reached 10^9/ml, the diameter of the halo was close to one-third of that of the plate, which was not suitable for distinguishing multiple fungal strains in one plate (Fig. 2B). Thus, in order to provide a direct observation of the chromogenic reaction results, 10^8/mL was selected as the optimal spore inoculum concentration.

Based on the above results, a plate chromogenic assay for rapid detection of the β-furanosidase-producing strains was developed. The complete procedures were as follows: 1 μl of the spore suspensions (10^8/mL) was spotted onto CD plates and inoculated at 30 °C for 5 h, where the distance between the spores of any two strains was not < 2 cm. Then the plates were overlaid with the GOD-POD bienzymatic system and carried out at 40 °C for 10 min. The formation of pink halos around the colonies can be used to evaluate the ability of the strains to produce β-fructofuranosidase.

3.2. Application of the plate chromogenic assay to evaluate the ability of the mutant strains to produce β-fructofuranosidase

In order to screen of strains with high ability to produce β-fructofuranosidase and synthesize FOS, a random mutant library of *A. niger* ATCC 20611 was constructed by UV mutagenesis. Totally, more than ten thousand of mutants were obtained. All mutants were spotted and detected on the CD plates according to the optimized plate chromogenic assay with the GOD-POD bienzymatic system. The diameters of the pink halos formed around the colonies were recorded. The mutant strains with larger pink halos than the parental strain ATCC 20611 were assigned to group A, and the remaining mutants were regarded as group B (data not shown). Finally, 13 mutants with larger pink halos from group A and 3 mutants randomly selected from group B were repeatedly determined with the plate chromogenic assay for the genetic stability. The chromogenic reaction results were shown in Fig. 3. The mutants with larger halos in group A were A1, A2, A3, A4, A5, A7, A8, A10, A11, A12, A13, A14 and A16, while the mutants with smaller halos in group B were A6, A9 and A15 (Fig. 3A, B). The diameters of the pink halos formed by A11 and A16 could reach 1.30 cm and 1.25 cm, respectively, which were more than twice larger than that of the parental strain ATCC 20611 (Fig. 3B, C). It could be supposed that A11 and A16 probably had higher ability to produce β-fructofuranosidase than other strains. In contrast, the halo diameters of A6, A9 and A15 were all <

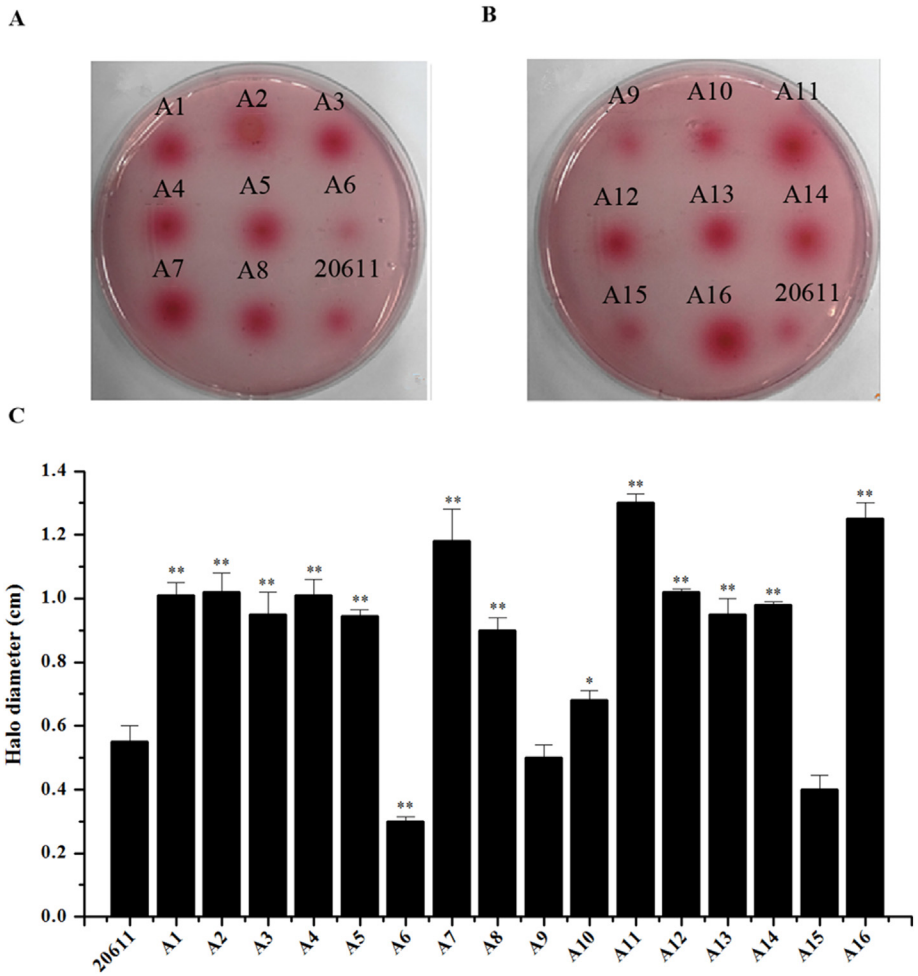

Fig. 3. Evaluation of the ability of mutant strains to produce β-fructofuranosidase by the plate chromogenic assay. (A), (B) 16 mutant strains (A1-A16) selected from a random mutant library of *A. niger* ATCC 20611 were cultivated onto CD plates for 5 h. The glucose released was detected by the GOD-POD bienzymatic system. ATCC 20611 was used as the parental strain. (C) The diameters of the halos formed around the colonies. Error bars represent standard deviation of three replicate cultures. Statistical analysis was performed by using Student's *t*-test. Significance at $P < .05$ or $P < .01$ was indicated by one or two asterisks, respectively.

0.55 cm (Fig. 3B, C). Particularly, the halo formed around the A6 was smallest (0.30 cm), indicating that the mutant A6 probably had the lowest ability to produce β-fructofuranosidase.

3.3. Determination of β-fructofuranosidase activity and FOS synthesis ability of the A. niger mutant strains

To further verify the correctness of the established plate chromogenic assay for screening of the β-fructofuranosidase-producing strains, the *A. niger* mutants selected above were used for fermentation and their β-fructofuranosidase activities were determined by DNS method. It was found that most of the mutants (A2, A3, A7, A11, A12, A13, A14 and A16) with larger halos than the parental strain ATCC 20611 displayed higher β-fructofuranosidase activity, and all the three mutants (A6, A9 and A15) with smaller halos exhibited lower β-fructofuranosidase activity (Fig. 4). Especially, A11 and A16 that showed the largest pink halos in the plate chromogenic assay possessed the highest enzyme activities (488 U/g and 452 U/g), which values were 1.65 fold and 1.52 fold than that of ATCC 20611 (296 U/g), respectively (Fig. 4). Meanwhile, the mutant A6 with the smallest halo in the plate assay exhibited the lowest enzyme activity (198 U/g), which was only 66.90% of the activity produced by ATCC 20611 (Fig. 4). Therefore, the ability of the mutants to produce β-fructofuranosidase could be reflected by the pink halos detected from the plate chromogenic assay method.

Furthermore, the mutants A11 (with the largest halo) and A6 (with the smallest halo) were evaluated for their abilities to synthesize FOS. These mycelia cells were added to high-concentration sucrose solution (50%, *w*/w) to perform the transglycosylation reaction and synthesize FOS. The transglycosilation products were identified by TLC (Fig. 5). It was discovered that kestose (GF2) and nystose (GF3) were the main components of FOS synthesized by the mycelia cells, however, the band of sucrose of the mutant A11 was significantly weaker than that of the parental strain ATCC 20611. As for 1^F-β-fructofuranosyl nystose (GF4), the band of GF4 of the muntant A11 was stronger than that of ATCC 20611 while the band of A6 was almost invisible. The FOS yield and composition produced by the mutants A11, A6 and the parental strain ATCC 20611 were shown in Table 1. During the transglycosylation reaction up to 4 h, the GF4 content for A6 was approx. 2.6%, which was only 36.9% of the GF4 content produced by ATCC 20611 (6.5%). Significantly higher GF4 level for A11 (11.2%) highlighted the enhancement in catalytic activity, as higher GF4 production was indicative of the strain converting sucrose more rapidly. In fact, the endpoint for a typical industrial FOS synthesis reaction was considered as the time when total FOS composition contained 10% GF4 (Trollope et al., 2015). A11 reached 10% GF4 in 4 h while it took the parental strain > 5 h to produce similar composition of FOS (Data not shown). This difference achieved at least 20% reduction in the time required to complete the reaction. Moreover, the amount of sucrose remaining in A11 (7.2%) was 36.8% less than that of the parental strain (11.4%), and the amount

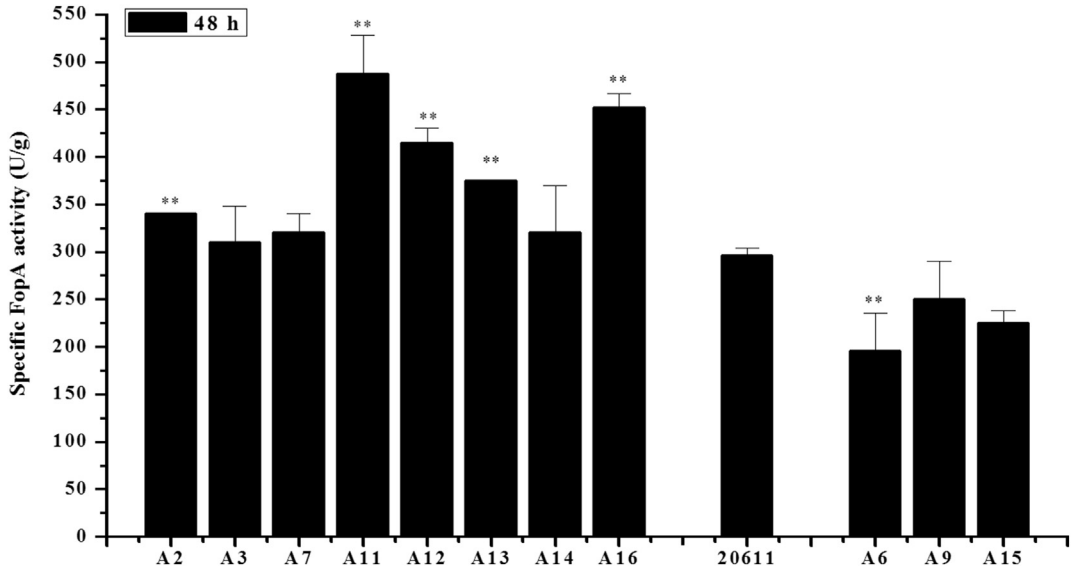

Fig. 4. Determination of β-fructofructofuranosidase activities of mutant strains by DNS method. 20,611 representing Aspergillus niger ATCC 20611 was used as the parental strain. Error bars represent standard deviation of three replicate cultures. Statistical analysis was performed by using Student's *t*-test. Significance at $P < .01$ was indicated by two asterisks.

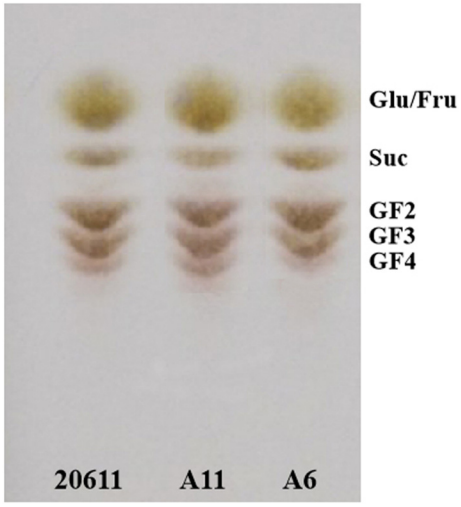

Fig. 5. TLC assay of the FOS composition formed by A11, A6 and ATCC 20611 during the transglycosylation reaction using 50% (*w*/w) sucrose as substrate. Glu, glucose; Fru, fructose; Suc, sucose; GF2: kestose; GF3: nystose; GF4: 1^F-β-fructofuranosyl nystose.

of sucrose remaining in A6 (14.7%) was 22.4% more than that of the parent strain, indicating that A11 and A6 consumed respectively more and less sucrose than ATCC 20611. As a result, the amount of total FOS produced by A11 (54.3%) was higher than that of ATCC 20611 (51.3%) and the FOS yield of A6 was lowest (50.1%). These results demonstrated that there was a positive correlation between halo diameter and FOS synthesis ability. A11 with the largest halo could be regarded as a potential high-yield strain for FOS synthesis while A6 with the smallest halo possessed low FOS synthesis ability. The superiority of the plate chromogenic assay in effective screening of strains with high β-fructofuranosidase activity for FOS production was worth promoting in FOS industry.

4. Discussion

During the last decades, the fructooligosaccharides (FOS) obtained from sucrose have attracted special attention due to their properties and functions. Hence, commercial production of FOS has been in the spotlight (Maiorano et al., 2008). The key enzyme in the FOS synthesis process (β-fructofuranosidase) can be produced by various microorganisms and its importance has prompted the development of a variety of methods for screening of microbial β-fructofuranosidase producers. Currently, common procedures for determining β-fructofuranosidase activity of a specific strain include strain fermentation, enzyme extraction, transglycosylation reaction and glucose concentration determination (Nascimento et al., 2016). These analysis procedures took too much time to identify the β-fructofuranosidase activity, so they were not suitable for high-throughput screening of sizeable strain samples. Under the circumstance, efforts have been taken to establish alternative methods for screening of β-fructofuranosidase producers (Reddy et al., 2010; Dominguez et al., 2006). Reddy et al. applied radial diffusion method to screen potent β-fructofuranosidase producers from

Table 1
FOS yield and composition produced by the β-fructofructofuranosidase from different strains[a].

Strain(s)	Sucrose (%)	Glu/Fru (%)[c]	GF2 (%)[d]	GF3 (%)[d]	GF4 (%)[d]	GFn (%)
A. niger ATCC 20611	11.4 (± 0.9)[b]	37.3 (± 1.2)	24.5 (± 0.8)	20.3 (± 1.1)	6.5 (± 0.2)	51.3 (± 3.3)
A11	7.2 (± 1.6)**	38.5 (± 2.2)	21.7 (± 3.2)	21.4 (± 4.6)	11.2 (± 2.3)*	54.3 (± 2.6)
A6	14.7 (± 1.2)**	35.2 (± 3.8)	28.7 (± 0.7)**	18.8 (± 1.4)	2.6 (± 0.1)**	50.1 (± 2.1)

[a] β-fructofructofuranosidase dosage was 6 U/g sucrose (substrate, 50% w/w) and the transglycosylation reaction was conducted at 50 °C for 4 h.
[b] Data in parentheses represent standard deviation of three replicate cultures. Statistical analysis was performed by using Student's *t*-test. Significance at $P < .05$ or $P < .01$ was indicated by one or two asterisks, respectively.
[c] Percentage of glucose and fructose in the reaction product.
[d] GF2: kestose; GF3: nystose; GF4: ^{1}F -β-fructofuranosyl nystose.

soil samples. In this method, triphenyl tetrazolium chloride (TTC) was used to indicate the presence of reducing sugars and the zone diameter was calculated to determine the hydrolytic activity of β-fructofuranosidases. However, this method was not be used to detect the β-fructofuranosidase activity of mycelium, and it is still necessary to additionally determine the transglycosylation activity of the crude enzyme. Due to the use of high-concentration sucrose (50% *w*/w) as substrate in the transglycosylation reaction, it was inferred that the plate chromogenic assay method proposed in this study has the potential to predict the transglycosylation activity of β-fructofuranosidase. The screen method established by Dominguez et al. (2006) was based on glucose oxidase-peroxidase reaction coupled with fructose dehydrogenase for determination of both glucose and fructose, and subsequent identification of the fungi with transfructosylation activity by the formation of pink (presence of glucose) and blue (presence of fructose) halos around the fungal colonies. But this method required a long incubation time of up to 72 h before determination of glucose and fructose. Moreover, the color overlapping and the dark background in the plate resulted in the halos around the colonies unclear enough to distinguish the difference. The plate chromogenic assay developed in this study was based solely on the GOD-POD bienzymatic system and applied for screening of an A. niger ATCC 20611 mutant library. Here, the amount of glucose released from sucrose was considered as the indicator and the clear pink halos around the colonies were used to evaluate the β-fructofuranosidase activity, so that β-fructofuranosidase-producing fungal strains could be screened out easily with the naked eye (Fig. 3), thus significantly reducing the screening workload. And the colony with the larger halo showed higher β-fructofuranosidase activity (Fig. 4) and FOS synthesis ability (Fig. 5), implying that this plate assay had the potential to predict the ability of strains to synthesize FOS. In addition, the screening process could be completed within only 5.5 h, which was significantly shorter than that required by the above-mentioned assay methods (Nascimento et al., 2016; Reddy et al., 2010; Dominguez et al., 2006). Therefore, the novel plate chromogenic assay method developed in this study can be advocated as more rapid and easily visible than the existing methods.

With a key objective of developing a rapid method for evaluation of the ability of Aspergillus species strains to produce β-fructofuranosidase, the suitable cultivation conditions for the plate chromogenic reaction were optimized. As shown in Fig. 1 and Fig. 2, the cultivation time and spore inoculum concentration of the strain can affect the chromogenic reaction. The concentration of 10^8/mL spore suspension inoculating on the plate for 5 h was the best condition. Besides, this study also determined the detection limit of the chromogenic reaction. The shortest cultivation time that could produce a clear pink halo was 2 h, and the minimum inoculum concentration when cultivation for 5 h was 10^7/mL (Fig. 2). It should be pointed out that control variable method was used to determine the optimal plate chromogenic assay conditions, so that the conditions here were conducted without considering interactions between cultivation time and spore inoculum concentration. Therefore, the method needs further optimization in the future application, especially for other fungi than Aspergillus species.

The effectiveness of the plate chromogenic assay was herein confirmed in a random mutant library of A. niger ATCC 20611 (Fig. 4). And the muant A11 whose β-fructofuranosidase activity was highest (488 U/g) among other strains was screened out from the library (Fig. 5). When A11 was used for industrial FOS production, it could display an enhanced catalytic activity by reducing the time to complete transglycosylation reaction. Thus, the required enzyme loading would be correspondingly reduced, which would contribute to improving industrial process economics. Moreover, in our previous research, this plate chromogenic assay method had been applied to screening of other Aspergillus species with the ability to produce FOS from a unique molasses habitat and an A. tubingensis strain, XG21, with great potential for commercial FOS production was successfully identified (Xie et al., 2017). According to these results, the new plate chromogenic assay method developed in this study would certainly be helpful in the rapid screening of Aspergillus species with high β-fructofuranosidase activity and most likely for other filamentous fungal species, which may improve the β-fructofuranosidase production by these fungi in the FOS industry.

In summary, based on this GOD-POD bienzymatic system, a rapid and effective plate chromogenic assay for screening of Aspergillus species with high β-fructofuranosidase activity for fructooligosaccharides production was established in this study. This method can reduce a large number of strains that need to be extensively screened to a smaller number of strains with the desired traits. Due to its simplicity, quickness and effectiveness, this plate chromogenic assay could be applied to screening of novel strains with high potential for FOS industrial-scale production.

Declaration of competing interest

None.

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