

Research Article

STUDY ON CULTURE CONDITIONS TO ENHANCE THE PRODUCTION OF α -GLUCOSIDASE INHIBITOR, ACARBOSE, FROM *Actinoplanes hulinensis* 1094

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ABSTRACT

Alpha-glucosidase inhibitors are a class of drugs that are widely used in the treatment of type 2 diabetes mellitus. Members of this class, such as acarbose, are pseudo-oligosaccharides that act as a competitive α -glucosidase inhibitors and have been widely used in the treatment of type 2 diabetes due to their ability to regulate postprandial blood glucose levels. They competitively inhibit enzymes that convert complex non-absorbable carbohydrates into simple absorbable carbohydrates. By delaying carbohydrate absorption, they reduce the rise in postprandial blood glucose. Acarbose has been shown to increase life expectancy in patients with type 2 diabetes mellitus and reduces the risk of the development of cardiovascular events in patients with impaired glucose tolerance. In this study, we aimed to enhance the biosynthesis of acarbose by optimizing the fermentation conditions of *Actinoplanes hulinensis* 1094. In 7 surveyed media (MT1, MT2, MT3, MT4, MT5, MT6, and MT7), the α -glucosidase inhibitory activity was highest in MT7 medium, the activity was 58.6%, the lowest was in MT6 medium. Component optimization of MT7 led to the formulation of an optimal medium containing (g/L): 50 maltose, 15 corn flour, 1 monosodium glutamate, 2 CaCl₂, 0.5 FeCl₃, 1.0 K₂HPO₄, and 2.5 CaCO₃. Subsequent optimization of physical conditions identified 30°C, pH 7.0, and 200 rpm as the most favorable parameters. Under these optimized conditions, the α -glucosidase inhibitory activity reached 82.2%, surpassing that of the 1 mg/mL standard acarbose control. These findings highlight the potential of *A. hulinensis* 1094 as a promising strain for high-yield acarbose production under cost-effective and scalable conditions.

Keywords: Acarbose, *Actinoplanes hulinensis* 1094, α -glucosidase, fermentation optimization, type 2 diabetes mellitus.

INTRODUCTION

Acarbose is an α -glucosidase inhibitor (AGI) derived from microbial fermentation, widely used in the treatment of type 2 diabetes mellitus. By reversibly inhibiting α -glucosidase enzymes in the small intestine, acarbose delays carbohydrate hydrolysis and reduces postprandial glucose levels without stimulating insulin secretion or causing hypoglycemia (Lee *et al.*, 2002; Liu and Ma, 2017). Its favorable safety profile makes it suitable for monotherapy or combination therapy with other oral antidiabetic agents.

Among AGIs, acarbose stands out as a successful secondary metabolite of *Actinoplanes* species. Various strategies have been explored to improve its biosynthesis. Wang *et al.* (2011) demonstrated that using maltose, glycerol, and monosodium glutamate as carbon and nitrogen sources enhanced acarbose production by *Actinoplanes utahensis* ZJB-08196, reaching a yield of 4288 mg/L (Wang *et al.*, 2011). Sun *et al.* (2012) further increased acarbose production to 6113 mg/L by supplementing S-adenosylmethionine (SAM) during fed-batch fermentation (Sun *et al.*, 2012). Similarly, Xue *et al.* (2013) reported that adding validamine reduced impurity C and improved acarbose yield to 6606 mg/L (Xue *et al.*, 2013).

Beyond carbon and nitrogen sources, environmental factors such as pH, temperature, and osmotic pressure play critical roles in optimizing fermentation. Optimal acarbose production has often been associated with pH 7.0, 27–30°C, and shaking speeds around 180 rpm (Wang *et al.*, 2011). Moreover, cost-effective media formulations using agricultural by-products

like corn flour and soybean meal have gained attention for industrial scalability.

Based on previous work that screened and evaluated the acarbose-producing strain *Actinoplanes hulinensis* 1094 (Do *et al.*, 2021), this study aims to optimize its culture medium and fermentation conditions to improve yield. This will be the scientific basis for further research oriented to develop an efficient and economically viable process for large-scale acarbose production, contributing to the sustainable manufacturing of AGIs for therapeutic use.

MATERIALS AND METHODS

Microorganism

The *A. hulinensis* 1094 was provided by the Enzyme Biotechnology Laboratory, Institute of Biology, Vietnam Academy of Science and Technology. The strain is registered on GenBank with the code MZ617258.1.

Media

The strain was stored on agar slant containing (g/L): 20 glucose, 5 peptone, 0.5 KCl, 1.0 K₂HPO₄, 0.5 MgSO₄, and 20 agar (pH 7.0). Propagation medium was composed of (g/L): 30 glucose, 40 soybean meal, 1.0 KH₂PO₄, 1 MgSO₄, and 20 CaCO₃ at pH 7. Initial fermentation medium (MT1) contained the following ingredients (g/L): 30 glucose, 30 maltose, 10 corn flour, 20 soybean meal, 1 monosodium glutamate, 2 CaCl₂, 0.5 FeCl₃, 1.0 K₂HPO₄, and 2.5 CaCO₃, pH 7.2. The *A. hulinensis* 1094 was cultured at 28°C, shaken at 180 rpm, for 144 hours (Wei *et al.*, 2010).

Chemicals

The chemicals used in the experiments were supplied by different companies: KH₂PO₄, MgSO₄, FeCl₃, CaCl₂, CaCO₃, monosodium

glutamate, and a thin layer of silica gel F254 was procured from Merck. Glucose, maltose, yeast extract, and peptone were purchased from ICN (USA). Acarbose, *p*-nitrophenyl- α -D-glucopyranoside (*p*NPG), and α -glucosidase (Intestinal acetone powders from rats) were purchased from Sigma (Louis, USA). Corn flour and soybean meal were purchased from Vietnam. All other chemicals are analytical grade, otherwise stated.

Screening of culture media for AGIs production

To evaluate the impact of different carbon

and nutrient sources on α -glucosidase inhibitor production, *A. hulinensis* 1094 was cultivated in seven distinct fermentation media (Wang *et al.*, 2011). Each medium contained a unique combination of components, as detailed in Table 1. After 144 hours, the fermented solution of *Actinoplanes* sp. was centrifuged at 4000 rpm in 15 min, followed by 12,000 rpm in 15 min. The supernatant was precipitated with ethanol in a ratio of sample/ethanol (1: 4; v/v) for 30 min, then centrifuged at 12,000 rpm in 10 min. The collected liquid was examined with thin layer chromatography (TLC) and α -glucosidase activity.

Table 1. Composition of the different fermentation media.

Components of the medium	MT1	MT2	MT3	MT4	MT5	MT6	MT7
Glucose	+	+	-	+	+	-	-
Maltose	+	-	+	+	+	-	+
Corn flour	+	+	+	+	-	+	+
Soybean meal	+	+	+	-	+	+	-
Glutamate	+	+	+	+	+	-	+
Mineral	+	+	+	+	+	+	+

Note: “-“: not using, “+“: using

Determination of α -glucosidase inhibitory activity

The α -glucosidase inhibitory activity was measured using *p*-nitrophenyl- α -D-glucopyranoside as the substrate, following the method described by Koh *et al.* (2018) with minor modifications. Briefly, 10 μ L of the test sample and 100 μ L of α -glucosidase (1 U/mL) in 0.1 M phosphate buffer (pH 6.9) were added to each well of a 96-well microplate, followed by pre-incubation at 25°C for 10 min. Subsequently, 50 μ L of 5 mM *p*-nitrophenyl- α -D-glucopyranoside

was added, and the reaction mixture was incubated for an additional 5 min (Koh *et al.*, 2018). Absorbance at 405 nm was measured before and after substrate addition. The inhibition rate was calculated as:

$$\text{Inhibition (\%)} = \frac{\Delta A(C) - \Delta A(S)}{\Delta A(C) \times 5} \times 100\%$$

Where, ΔA represents the change in absorbance before and after substrate incubation, C is the control, and S is the sample. All experiments were performed in triplicate.

Thin layer chromatography

TLC was performed to qualitatively detect acarbose in culture supernatants. A volume of 10 μ L of each culture sample and standard acarbose solution (1 mg/mL) was spotted onto a silica gel 60 F254 TLC plate (Merck, 0.25 mm thickness). The plate was developed using a solvent system of *n*-butanol: ethanol: water (5:3:2, v/v/v) as described by Goeke *et al.* (1996). After development, the plate was dried, sprayed with 10% sulfuric acid in ethanol, and heated at 120°C for 15 min to visualize carbohydrate bands. A positive result was defined as the appearance of a band at the same retention factor (R_f) value as the acarbose standard (Goeke *et al.*, 1996).

Effect of time on the production of AGIs

The *A. hulinensis* 1094 was cultured in MT7 medium. Survey time from 24 hours to 192 hours. Every 24 hours of culture, 2 mL of the culture was collected to determine α -glucosidase inhibitory activity and to examine AGIs biosynthesis by TLC (Wang *et al.*, 2011).

Optimizing corn flour and maltose concentration on the biosynthesis of AGIs

Optimizing the maltose source concentration, the *A. hulinensis* 1094 strain was cultured in MT7 medium in which the maltose source was at different concentrations: 2, 3, 4, 5, and 6% (w/v).

To investigate the effect of corn flour on acarbose biosynthesis, the *A. hulinensis* 1094 strain was cultured in MT7 medium. In which corn flour is added at different concentrations: 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5% (w/v) (Wang *et al.*, 2011; Choi and Shin, 2003).

Effect of pH, temperature, and shaking speed during culture

To determine the optimal initial medium pH, temperature, and shaking speed for culture (Wang *et al.*, 2011; Wei *et al.*, 2010), the *A. hulinensis* 1094 strain was cultured in MT7 fermentation modified medium for 144 hours under the following research conditions: shaking speed of 100, 180, 200 and 220 rpm; temperature of 25, 28, and 30°C; initial pHs adjusted to 6.0, 6.5, 7.0, 7.2, 8.0, and 8.5 with either 1 N NaOH or 1 N HCl, in which the best parameters of the previous study were selected for the following studies. The optimal shaking speed, temperature and pH for high acarbose biosynthesis were determined based on TLC chromatograms and α -glucosidase inhibitory activity.

Data analysis

The results were measured using an ELISA reader at a wavelength of 405 nm. The experiments were repeated three times independently, and the results were presented as average values; the results were processed and illustrated with Microsoft Excel 2016 software.

RESULTS AND DISCUSSION

Screening of culture media for AGIs production

The compositions of media MT1–MT7 were designed based on previous studies of acarbose-producing *Actinoplanes* strains, such as *A. utahensis* and *Actinoplanes* sp. (Wang *et al.*, 2011; Wei *et al.*, 2010). These media differed in their inclusion or exclusion of key carbon and nitrogen sources with the goal of identifying critical nutrients that

influence α -glucosidase inhibitor production in *A. hulinensis* 1094.

After 144 hours of fermentation at 28°C and 180 rpm, AGIs activity varied notably among the media. MT7 exhibited the highest α -glucosidase inhibitory activity (58.6%), followed by MT1 (54.6%), MT4 (42.7%), and MT3 (44.3%) (Figure 1A). Importantly, all four of these media contained both maltose and corn flour, suggesting that these two components play a decisive role in enhancing acarbose biosynthesis. TLC further confirmed the presence of acarbose-like compounds in MT1, MT4, and MT7, as evidenced by bands

corresponding to the R_f value of standard acarbose ($R_f = 0.4$) (Figure 1B).

These findings are consistent with prior studies demonstrating the crucial role of maltose as a direct precursor in acarbose biosynthesis (Choi and Shin, 2003; Li *et al.*, 2022) and the beneficial contribution of corn-based substrates to strain growth and product formation (Li *et al.*, 2012; Wei *et al.*, 2010; Weng *et al.*, 2020). This initial screening thus supports the hypothesis that maltose and corn flour are key ingredients for AGIs production and provides a rationale for selecting MT7 as the base for further optimization.

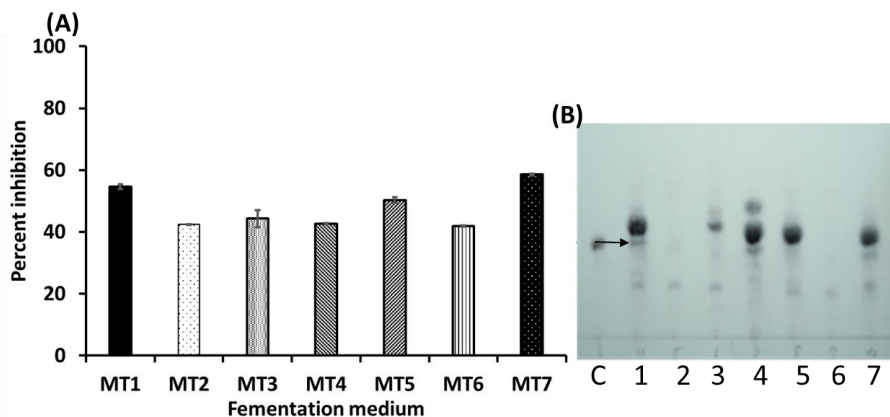


Figure 1. Screening of culture media for AGIs production from *A. hulinensis* 1094. (A) Percentage of α -glucosidase inhibition of *A. hulinensis* 1094 cultured on 7 different media. (B) Results of α -glucosidase inhibition assessment by TLC (Lanes 1–7 correspond to MT1, MT2, MT3, MT4, MT5, MT6, and MT7 medium, respectively; Lane C: acarbose with concentration of 1 mg/mL).

Effect of fermentation time on AGIs production

To determine the optimal fermentation duration for acarbose biosynthesis, *A. hulinensis* 1094 was cultured in MT7 medium and sampled at 24-hour intervals over a period of 192 hours. As shown in Figure 2A, α -glucosidase inhibitory activity remained negligible during the initial 24 hours, began to increase notably after 48 hours, and peaked at 144 hours with a

maximum inhibition of 57.9%. TLC analysis (Figure 2B) confirmed the presence of acarbose-like compounds from 48 hours onward, with the most intense band observed at 144 hours with R_f value of 0.4 corresponding to the acarbose standard concentration of 1 mg/mL. Beyond this point (168–192 hours), the inhibitory activity slightly declined and TLC revealed the emergence of additional side products with an R_f value of 0.24 (Figure 2B).

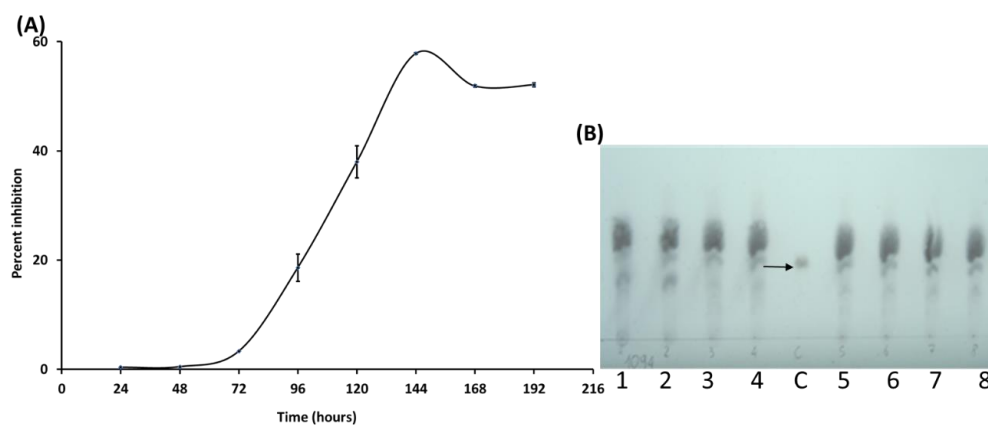


Figure 2. Effect of time culture on AGIs production from *A. hulinensis* 1094. (A) Percentage of α -glucosidase inhibition of *A. hulinensis* 1094 cultured at different times. The values are mean \pm SD. (B) TLC chromatograms of acarbose samples of *A. hulinensis* 1094 under different fermentation times (Lanes 1–8, samples collected from 24–192 hours, respectively; Lane C: acarbose with a concentration of 1 mg/mL).

These results indicate that 144 hours is the most suitable fermentation duration for maximizing acarbose biosynthesis while minimizing by-product formation. The observed biosynthetic pattern is consistent with previous reports. For example, Wei *et al.* (2010) found that *Actinoplanes* sp. A56 produced 1043 mg/L acarbose at 144 hours (Wei *et al.*, 2010), while Wang *et al.* (2011) reported a yield of 4210 mg/L from *A. utahensis* ZJB-08196 under similar conditions (Wang *et al.*, 2011). Taken together, this suggests that the 144-hour fermentation period is commonly associated with optimal metabolic activity for acarbose-producing *Actinoplanes* strains.

Therefore, in subsequent optimization experiments, 144 hours was selected as the standard cultivation time for evaluating the effects of medium components and physical parameters on acarbose production.

Effect of maltose concentration on the biosynthesis of AGIs

To determine the optimal maltose concentration for acarbose biosynthesis, *A.*

hulinensis 1094 was cultured in MT7 medium supplemented with varying maltose levels (2–6%; w/v), while other components remained constant. As shown in Figure 3A, α -glucosidase inhibitory activity increased progressively with maltose concentration. When a maltose concentration increased to 5%, α -glucosidase inhibitory activity reached its highest level (63.2%), and decreased to 49.2% at a maltose concentration of 6% (Figure 3A). TLC analysis (Figure 3B) supported these findings, with the most intense acarbose band and minimal by-product formation observed at 5%.

These results indicate that 5% maltose is optimal for acarbose biosynthesis in this strain, consistent with previous reports. Wang *et al.* (2011) demonstrated that *A. utahensis* ZJB-08196 produced the highest acarbose yield (4288 mg/L) when cultured with 50 g/L maltose (~5%). The production acarbose efficiency strongly reduced when maltose increased up to 60 to 80 g/L (Wang *et al.*, 2011). Similarly, Choi and Shin (2003) reported that maintaining high

maltose concentrations during fermentation resulted in optimal acarbose biosynthesis, which correlated with an osmotic pressure of ~500 mOsm/kg—a condition also achieved using 50 g/L maltose (Choi and Shin, 2003).

At the molecular level, maltose has been identified as a direct precursor in the acarbose biosynthetic pathway (Lee *et al.*, 1997), further confirming its critical role.

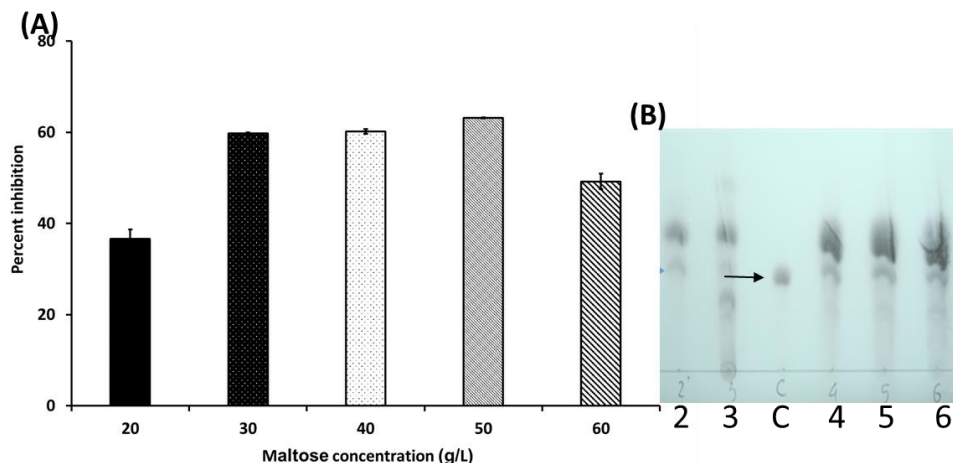


Figure 3. Effect of maltose concentration on the production of AGIs from *A. hulinensis* 1094. (A) Effect of maltose concentration on the α -glucosidase inhibitory. (B) TLC chromatograms of biosynthetic acarbose samples from *A. hulinensis* 1094 after optimization of maltose concentration (Lanes 2–6: 20–60 g/L of maltose concentration; Lane C: acarbose with concentration of 1 mg/mL).

Effect of corn flour concentration on AGIs production

Corn flour was evaluated as a low-cost nutrient source for enhancing acarbose biosynthesis due to its availability and its content of both carbohydrates and organic nitrogen. To assess its impact, *A. hulinensis* 1094 was cultured in MT7 modified medium with a maltose concentration of 50 g/L and supplemented with varying concentrations of corn flour (0–25 g/L), while other conditions were kept constant.

As shown in Figure 4A, α -glucosidase inhibitory activity was increased from 25.1 to 62.7%, when supplemented with corn flour concentrations of 0–15 g/L. When the corn flour concentration was increased to 20 g/L, α -glucosidase inhibitory activity

decreased to 49.8% and continued to decrease by 48% at a concentration of 25 g/L. Thus, the α -glucosidase inhibitory activity was highest at a corn flour concentration of 15 g/L, activity reached of 62.7%. TLC analysis (Figure 4B) confirmed these findings, with the most intense acarbose band and minimal by-product formation observed at 15 g/L corn flour. Corn flour was an important nitrogen source for many microorganisms (Shah and Cheryan, 1995; Silveira *et al.*, 2001; Vu and Kim, 2009). Wei *et al.* (2010) found that corn flour was an important factor for acarbose production and used for the industrial-scale production of acarbose by *Actinoplanes* sp. A56 (Wei *et al.*, 2010). The results showed that moderate corn flour levels provide an optimal balance of nutrients for acarbose biosynthesis. At low concentrations (0–10 g/L), limited

nutrient availability may restrict metabolic flux, while excessive corn flour (≥ 20 g/L) may introduce osmotic stress or shift metabolism toward alternative pathways, reducing product specificity.

These findings align with previous reports that corn flour enhances acarbose production

not only as a carbon source but also by supporting filamentous growth in *Actinoplanes* species (Wang *et al.*, 2011). Based on both inhibitory activity and TLC analysis, 15 g/L corn flour was selected as the optimal concentration for further experiments.

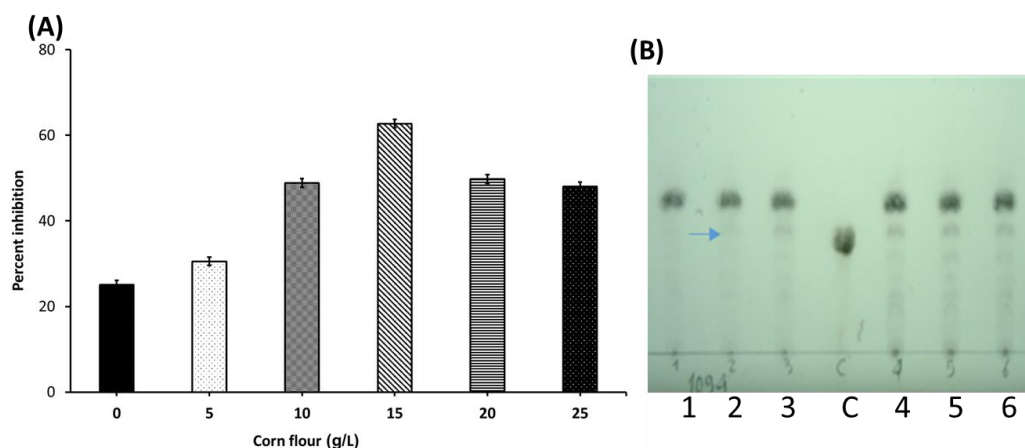


Figure 4. Effect of corn flour concentration (0–25 g/L) on the production of AGIs from *A. hulinensis* 1094. (A) Effect of corn flour on the α -glucosidase inhibitor. Values are expressed as mean \pm SD ($n = 3$). (B) TLC chromatograms of biosynthesized acarbose samples from cultures supplemented with different corn flour concentrations (Lanes 1–6 correspond to 0, 5, 10, 15, 20, and 25 g/L, respectively; Lane C: acarbose with a concentration of 1 mg/mL).

Effect of initial pH on AGIs production

The initial pH of the culture medium plays a critical role in microbial metabolism and secondary metabolite biosynthesis. To evaluate its effect on acarbose production, *A. hulinensis* 1094 was cultivated in MT7 modified medium with the different initial pH. As shown in Figure 5, the highest inhibitory activity was observed at pH 7.0 reaching 69.5% and decreased at higher pH with the inhibitory activity reaching 31% at the initial pH of 8.5. The data showed that neutral conditions are optimal for acarbose

biosynthesis in this strain. These results were in strong agreement with previous studies by *Actinoplanes* sp. For example, *A. utahensis* ZJB-08196 produced the highest acarbose yield at pH 7.0 (Wang *et al.*, 2011), while *Actinoplanes* sp. A56 and SN 223/29 also exhibited maximum biosynthesis at pH 7.2 (Lee *et al.*, 1997; Wei *et al.*, 2010). Choi and Shin (2003) similarly reported optimal production at neutral pH for strain CKD485-16 (Choi and Shin, 2003). Collectively, these results showed that pH 7.0 was the highest optimum for acarbose production by *A. hulinensis* 1094.

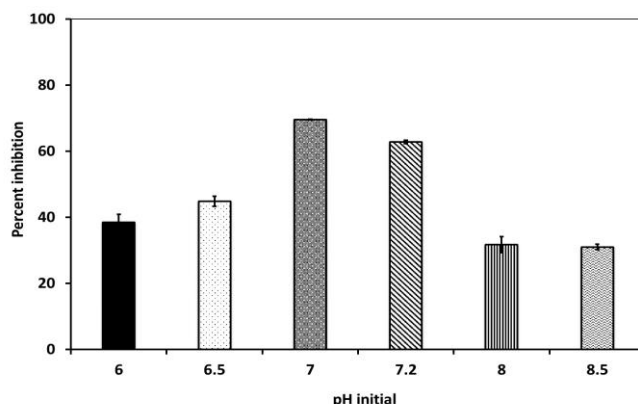


Figure 5. Effect of pH medium on the α -glucosidase inhibitory activity.

Effect of culture temperature on AGIs production

As shown in Figure 6A, the highest inhibitory activity (75.5%) was achieved at 30°C, indicating this as the optimal temperature for acarbose production by *A. hulinensis* 1094. TLC analysis (Figure 6B) supported these results, with the strongest acarbose band and minimal by-products observed at this temperature. These results

were consistent with previous reports on *Actinoplanes* sp. strains. For instance, *A. utahensis* ZJB-08196 produced the highest yield at 27°C (Wang *et al.*, 2011), while strains CKD485-16 and SN 223/29 showed optimal biosynthesis around 28°C (Choi and Shin, 2003; Lee *et al.*, 1997). Based on both α -glucosidase inhibitory activity and the ability to produce acarbose, 30°C was selected as the optimal temperature for subsequent fermentation trials.

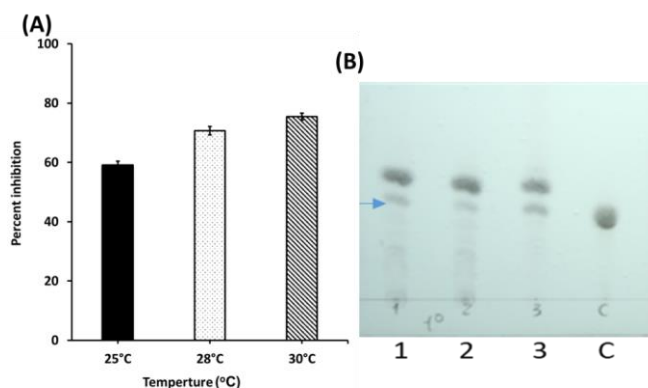


Figure 6. Effect of culture temperature on AGIs production by *A. hulinensis* 1094. (A) Effect of incubation temperature on the α -glucosidase inhibitory activity. (B) TLC chromatograms of biosynthesized acarbose samples at different temperatures. Lanes 1–3 represent 25 °C, 28 °C, and 30 °C, respectively; Lane C: acarbose with a concentration of 1 mg/mL.

Effect of shaking speed on AGIs production

Agitation rate plays a vital role in aerobic fermentation by influencing oxygen transfer, nutrient mixing, and morphological

development of filamentous microorganisms. As illustrated in Figure 7A, α -glucosidase inhibitory activity was highest at 200 rpm (81.3%), significantly outperforming both lower (100–180 rpm: 43.0–47.3%) and higher speeds (220 rpm: 43.3%). Notably, the activity at 200 rpm even exceeded that of the 1 mg/mL standard acarbose control (72.8%), indicating efficient biosynthesis under this condition. The results of our study are also consistent with Wang *et al.* 2011. The authors investigated the effect of different shaking speeds on the acarbose biosynthesis ability of *A. utahensis* ZJB-08196 and also showed that a rotation speed of 200 rpm was advantageous to *A. utahensis* ZJB-08196.

When shaking at a speed of 230 rpm, acarbose production ability decreased significantly (Wang *et al.*, 2011). Hence, the possible decline at 220 rpm may result from excessive shear stress disrupting cell integrity or filamentous morphology, which is essential for metabolite production in *Actinoplanes* sp.

TLC analysis (Figure 7B) further confirmed the specificity of product formation at 200 rpm, with a clear acarbose band and minimal side products. These findings are consistent with previous studies, where intermediate agitation promoted optimal balance between oxygenation and shear tolerance in *Actinoplanes* fermentation (Wang *et al.*, 2011).

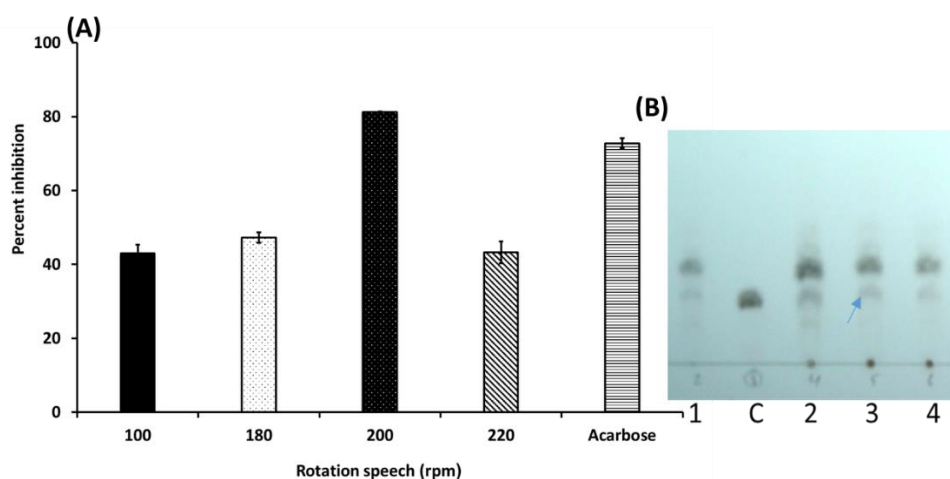


Figure 7. Effect of agitation speed on the AGIs production by *A. hulinensis* 1094. (A) Effect of agitation speed on the α -glucosidase inhibitor. (B) TLC chromatograms of production acarbose samples from *A. hulinensis* 1094 after optimization of agitation speed (Lanes 1–4: shaking speeds of 100, 180, 200, and 220 rpm, respectively; Lane C: acarbose (Sigma) with a concentration of 1 mg/mL).

Overall, the AGIs production by *A. hulinensis* 1094 was most effective in the optimum medium (g/L) containing: 50 maltose, 15 corn flour, 1.0 monosodium glutamate, 2 CaCl₂, 0.5 FeCl₃, 1.0 K₂HPO₄, and 2.5 CaCO₃ at 30°C with initial pH of 7.0,

and 200 rpm for 144 hours. The α -glucosidase inhibitory activity of *A. hulinensis* 1094 in the culture condition before and after optimization was presented in Figure 8. This inhibitory activity increased 1.4 times after optimization.

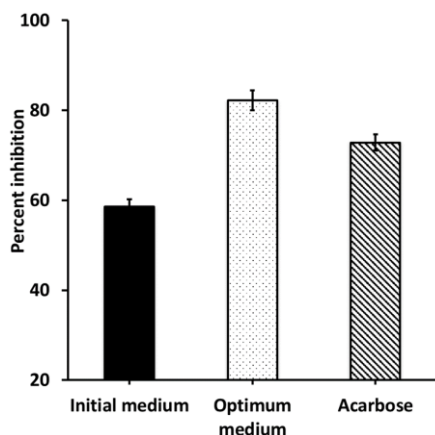


Figure 8. Results of determination of α -glucosidase inhibitory activity of fermentation broth and acarbose with a concentration of 1 mg/mL.

CONCLUSION

The effects of the nutrient components and cultural parameters on the α -glucosidase inhibitor production by *A. hulinensis* 1094 were investigated. Through systematic optimization of medium components and fermentation parameters, the optimal conditions for acarbose biosynthesis were established. The most effective medium consisted of (g/L): 50 maltose, 15 corn flour, 1 monosodium glutamate, 2 CaCl₂, 0.5 FeCl₃, 1.0 K₂HPO₄, and 2.5 CaCO₃. Under these conditions, the strain exhibited its highest biosynthetic performance after 144 hours of cultivation at 30°C, pH 7.0, and 200 rpm shaking speed. The α -glucosidase inhibitory activity reached 82.2%, representing a 1.4-fold improvement compared to the pre-optimized condition (58.6%), and even exceeding the inhibitory effect of standard acarbose at 1 mg/mL (72.8%).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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