SHORT COMMUNICATION

A novel osmolality-shift fermentation strategy for improving acarbose production and concurrently reducing byproduct component C formation by *Actinoplanes* sp. A56

Xin Cheng · Wei-fu Peng · Lin Huang · Bao Zhang · Kun-tai Li

Abstract Component C (Acarviosy-1,4-Glc-1,1-Glc) was a highly structural acarbose analog, which could be largely formed during acarbose fermentation process, resulting in acarbose purification being highly difficult. By choosing osmolality level as the key fermentation parameter of acarbose-producing *Actinoplanes* sp. A56, this paper successfully established an effective and simplified osmolality-shift strategy to improve acarbose production and concurrently reduce component C formation. Firstly, the effects of various osmolality levels on acarbose fermentation were firstly investigated in a 50-l fermenter. It was found that 400–500 mOsm/kg of osmolality was favorable for acarbose biosynthesis, but would exert a negative influence on the metabolic activity of *Actinoplanes* sp. A56, resulting in an obviously negative increase of acarbose and a sharp formation of component C during the later stages of fermentation (144–168 h). Based on this fact, an osmolality-shift fermentation strategy (0–48 h: 250–300 mOsm/kg; 49–120 h: 450–500 mOsm/kg; 121–168 h: 250–300 mOsm/kg) was further carried out. Compared with the osmolality-stat (450–500 mOsm/kg) fermentation process, the final accumulation amount of component C was decreased from 498.2 ± 27.1 to 307.2 ± 9.5 mg/l, and the maximum acarbose yield was increased from $3,431.9 \pm 107.7$ to $4,132.8 \pm 111.4$ mg/l.

Keywords *Actinoplanes* sp. A56 · Acarbose · Component C · Osmolality-shift fermentation strategy

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Introduction

Acarbose, a pseudotetrasaccharide, is one of the most important α-glucosidase inhibitors that have been clinically and widely used in the treatment of type II diabetes mellitus to control the blood sugar contents of patients after meals [13]. As the member of the C7N-aminocyclitol family of natural products, acarbose is composed of an aminocyclitol and valienamine via a nitrogen bridge to C-4 of a 6-deoxy-D-glucose, in which pseudodisaccharide acarviosine (valienaminyl-4-amino-4,6-dideoxyglucose) serves as the active pharmacophore responsible for the inhibition of intestinal α-glucosidase and sucrase [9, 10].

The genus of *Actinoplanes* was the major microorganism for acarbose production, such as the reported *Actinoplanes* sp. SE50/110 [16, 19], *Actinoplanes* sp. CKD485-16 [2], *Actinoplanes* sp. SN223/29 [6], and *Actinoplanes utahensis* ZJB-08196 [4]. Until the year 2012, the complete genome sequence of *Actinoplanes* sp. SE50/110 had been successfully illuminated by Schwientek et al. [12], and it was the first publicly available genome of the genus *Actinoplanes*. Additionally, *Streptomyces glaucescens* GLA.O was identified as a second strain possessing a gene cluster (*gac*-cluster) for acarbose biosynthesis, and the *gac* cluster exhibited high similarities to the *acb* gene cluster from *Actinoplanes* [11]. To date, there were many reports available on how to improve acarbose production by implementing various fermentation strategies, such as the medium optimization [14, 18], osmolality control [1, 5], and the performance of fed-batch fermentation [15]. Although the acarbose productivity was largely improved with the application of these fermentation strategies, it should be noted that the synthesis of a series of acarbose analogs was often accompanied by the fermentation process of *Actinoplanes* sp., especially the large-scale formation of component C

(acarviosy-1,4-Glc-1,1-Glc) [17]. Due to its highly structural similarity to acarbose molecule, a large amount of component C would not only make the acarbose purification become difficult but also reduce the product quality. Therefore, it is an attractive proposition to reduce and even eliminate component C formation during the fermentation process of acarbose.

Our previous studies had been detailedly focused on the scale-up and optimization of industrial acarbose fermentation by *Actinoplanes* sp. A56 in a 30,000-l fermenter, and approximately 5,000 mg/l of acarbose was obtained [7, 8]. However, the concentration of component C could highly reach 530 mg/l at the end of fermentation. To improve acarbose production and concurrently reduce byproduct component C formation, the present work chose osmolality level as the key fermentation parameter to investigate its effects on the metabolic process of *Actinoplanes* sp. A56, and then an effective and simplified osmolality-shift control strategy was successfully established.

Materials and methods

Microorganism and media

Actinoplanes sp. A56 was used for acarbose production throughout this study, which was maintained on agar slant containing (g/l): glucose, 20; peptone, 5; KCl 0.5; K_2HPO_4 1.0; $MgSO_4$ 0.5; agar 20; pH 7.0.

Seed medium was composed of (g/l): starch, 10; glucose, 20; corn steep liquor (CSL), 20; soy bean flour, 10; KH_2PO_4, 1.0; $MgSO_4$, 1.0; $CaCO_3$, 2.0. The pH was adjusted to 7.0–7.2 with 1 mol/l of NaOH before autoclaving.

Fermentation medium contained the following ingredients (g/l): starch, 30; glucose, 50; CSL, 10; soy bean flour, 20; monosodium glutamate, 1.0; $FeCl_3$, 0.5; K_2HPO_4 1.0; $CaCO_3$ 2.0. The initial pH value was adjusted to 7.2–7.4 with 1 mol/L of NaOH prior to sterilization.

Feed medium for the fed-batch fermentation in 50-l fermenter was as follows (g/l): maltose 300; glucose, 100.

Cultivation conditions

The fermentation of *Actinoplanes* sp. A56 was performed in a 50-l stirred bioreactor equipped with a temperature probe, pH probe (Mettler Toledo) and dissolved oxygen (DO) probe (Mettler Toledo). Firstly, the preculture was carried out in a 1,000-ml shake flask containing 300 ml of sterile seed medium inoculated with cells from four fresh slants, and cultivated at 28 °C on a rotary shaker at 180 rpm for 48 h. Then, the seed culture (3,000 ml) was transferred into the 50-l fermenter with 30 l of fermentation medium. The fermentation conditions were controlled as follows:

28 °C of cultivation temperature; 7.0–7.2 of pH value through automatic feedback with 1 mol/l of NaOH or HCl; 40–50 % of DO concentration by adjusting agitation speed and airflow rate; 168 h of time cycle.

To investigate the effects of osmolality levels on *Actinoplanes* sp. A56 fermentation, the required osmolalities were associated with the total sugar concentrations in broths by adjusting the feeding rate of feed medium, as described in our previous report [7].

Analytical methods

Cell biomass was quantified with dry cell weight (DCW). The concentrations of acarbose and component C in fermentation broths were determined using HP1100 HPLC system (Agilent), as reported previously [8]. The osmolality of the fermentation broth was measured by the determination of freezing point depression.

All assays were performed in triplicate, and the results were presented as mean ± SD (standard deviation).

Results and discussion

Effects of various osmolality levels on the fermentation processes of Actinoplanes sp. A56

For an investigation on the effect of osmolality on the fermentation process of *Actinoplanes* sp. A56, the osmolality levels of fermentation broths were controlled at 250–300, 350–400, 450–500, and 550–600 mOsm/kg, respectively. Table 1 summarizes the time courses of cell growth, acarbose production and component C formation.

As shown in Table 1, a higher osmolality level would exert a negative influence on the cell growth of *Actinoplanes* sp. A56. Furthermore, compared to the fermentation processes with 250–300 and 350–400 mOsm/kg of osmolality levels, a higher osmolality level (450–500 and 550–600 mOsm/kg) would cause *Actinoplanes* sp. A56 mycelium prematurely entry into the autolysis period (at 144 h).

Maltose was a direct precursor incorporated into acarbose molecule, and it was reported that a relatively higher osmolality level could accelerate the intracellular transport of maltose, resulting in an increase of acarbose production [3]. The existing literatures revealed that 400–500 mOsm/kg of osmolality was favorable for acarbose production by *Actinoplanes*, and both lower and higher osmolalities had severely negative effects [17]. Similarly, our research showed that although 250–300 mOsm/ kg was the most advantageous osmolality level for cell growth of *Actinoplanes* sp. A56, the lowest acarbose yield (2,350.3 ± 60.3 mg/l) was obtained, as shown in Table 1. When the osmolality concentration was controlled at

Table 1 Time courses of cell growth, acarbose production and component C formation during the fermentation processes of *Actinoplanes* sp. A56 with various osmolality control levels

| Parameters	Osmolality (mOsm/kg)	Results at different fermentation times							
		0	24	48	72	96	120	144	168
DCW (g/l)	250–300	5.7 ± 0.2	10.4 ± 0.2	20.1 ± 0.5	24.7 ± 0.7	26.8 ± 0.7	28.5 ± 1.1	30.2 ± 0.6	31.4 ± 0.5
	350–400	5.5 ± 0.3	9.3 ± 0.4	18.9 ± 0.8	24.5 ± 0.9	26.0 ± 1.1	27.4 ± 0.3	29.0 ± 0.6	30.1 ± 0.4
	450–500	5.7 ± 0.4	9.1 ± 0.6	16.4 ± 0.5	22.8 ± 0.8	23.9 ± 0.6	25.7 ± 1.2	27.2 ± 0.9	26.0 ± 0.5
	550–600	5.6 ± 0.1	8.8 ± 0.5	16.0 ± 0.2	21.6 ± 0.8	23.7 ± 1.1	24.4 ± 0.7	24.6 ± 0.9	22.8 ± 0.8
Acarbose (mg/l)	250–300			356.0 ± 18.2	967.5 ± 36.7	1,624.4 ± 51.1	1,935.7 ± 62.0	2,198.6 ± 76.9	2,350.3 ± 60.3
	350–400			385.6 ± 22.5	1,024.4 ± 49.3	2,095.1 ± 68.8	2,437.2 ± 88.1	2,808.6 ± 50.6	2,942.3 ± 89.3
	450–500			403.9 ± 19.7	1,225.5 ± 51.8	2,581.0 ± 87.5	3,167.6 ± 86.1	3,370.8 ± 78.4	3,285.4 ± 104.2
	550–600			342.3 ± 15.6	893.4 ± 37.1	1,688.2 ± 64.1	2,056.7 ± 50.5	2,629.4 ± 66.0	2,444.1 ± 73.4
Component C (mg/l)	250–300			1.7 ± 0.2	3.2 ± 0.2	22.2 ± 1.0	55.4 ± 3.9	156.5 ± 8.1	246.3 ± 12.9
	350–400			2.3 ± 0.1	4.2 ± 0.3	21.8 ± 0.6	61.1 ± 5.4	190.3 ± 13.9	282.4 ± 15.7
	450–500			2.0 ± 0.2	5.7 ± 0.3	25.8 ± 1.5	93.1 ± 8.8	214.2 ± 11.6	515.8 ± 18.8
	550–600			1.8 ± 0.1	5.2 ± 0.2	26.8 ± 1.4	116.1 ± 10.1	261.8 ± 14.8	568.2 ± 13.7

450–500 mOsm/kg, the maximum acarbose titer reached 3,370.8 ± 78.4 mg/l at 144 h, which was significantly higher than those obtained in the other three fermentation processes. However, it was noteworthy that an obvious upward trend of component C could be observed along with the increases of osmolalities, as also shown in Table 1. Additionally, the component C concentrations under the relatively higher osmolalities (450–500 and 550–600 mOsm/kg) were sharply increased from 214.2 ± 11.6 (144 h) to 515.8 ± 18.8 mg/l (168 h) and 261.8 ± 14.8 (144 h) to 568.2 ± 13.7 mg/l (168 h), respectively.

In the acarbose-producing *Actinoplanes* sp. CKD485-16, Choi and Shin [2, 3] found that excess component C was produced in later stages of acarbose fermentation, which was derived directly from acarbose with a novel intracellular glucosyltransferase. For *Actinoplanes* sp. A56, a higher osmolality level would exert a negative influence on the specific activities of the key carbon metabolism enzymes (data not shown), resulting in a premature cell autolysis. And it was just during this autolysis period that an obviously negative increase of acarbose and a sharp rise in component C formation were synchronously appeared. As a hypothesis, the enormous component C accumulation was due to the severe decline of the metabolic activities of *Actinoplanes* sp. A56. Thus, it would probably be an effective measure to decrease component C formation by improving the metabolic activities of *Actinoplanes* sp. A56 at the later stages of fermentation.

Effects of osmolality-shift strategy on acarbose biosynthesis and component C formation

As mentioned above, when the fermentation of *Actinoplanes* sp. A56 was performed under a relatively high osmolality, the decline of metabolic activities would result in an obvious decrease of acarbose production together with a sharp increase of component C formation during the later stages of fermentation (144–168 h). To improve acarbose production and simultaneously avoid excessive synthesis of component C, an osmolality-shift strategy was further carried out for *Actinoplanes* sp. A56 fermentation in 50-l fermenter, in which the osmolality levels were controlled at 250–300 (0–48 h), 450–500 (49–120 h) and 250–300 mOsm/kg (121–168 h), respectively.

Figure 1 shows the time profiles of osmolality levels, cell growth, acarbose production and component C formation under the two fermentation processes with the osmolality-shift and osmolality-stat (450–500 mOsm/kg) control strategies. As expected, a significant improvement of the cell growth condition was observed using the osmolality-shift strategy. As shown in Fig. 1b, the maximum DCW was up to 29.3 ± 0.9 g/l, which was obviously higher than that (26.8 ± 0.7 g/l) obtained in the case of osmolality-stat
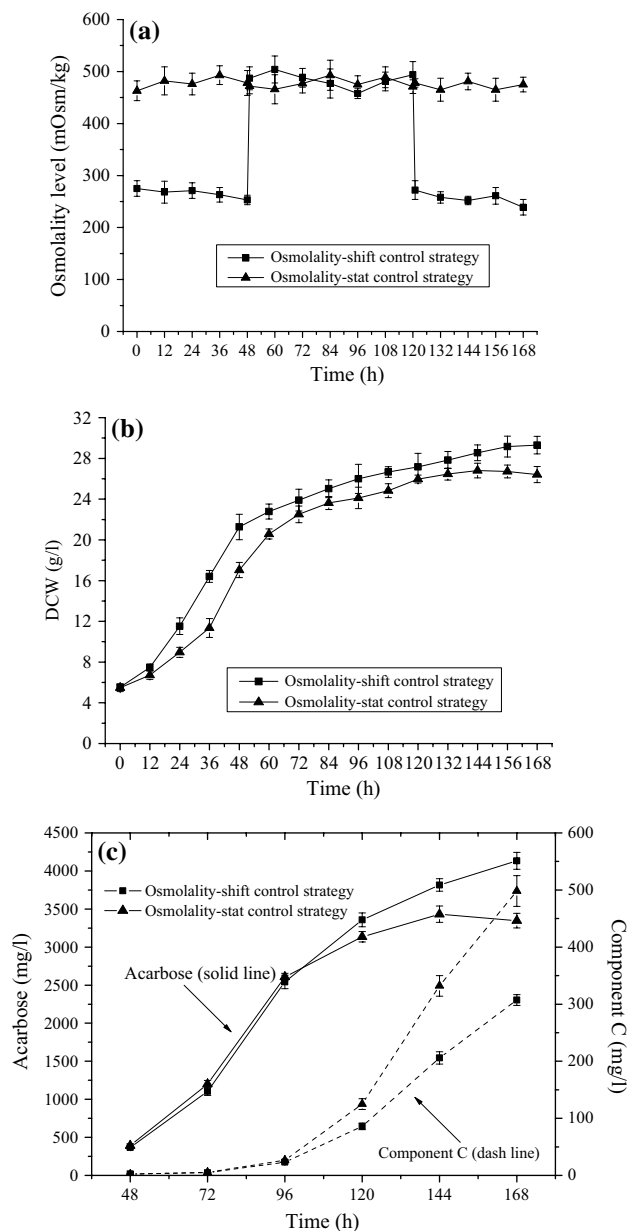
Fig. 1 Time courses of osmolality levels (**a**), cell growth (**b**), acarbose production (**c**) and component C formation (**c**) during the fermentation processes of *Actinoplanes* sp. A56 with the osmolality-shift and osmolality-stat control strategies

fermentation. More importantly, the autolysis phenomenon of *Actinoplanes* sp. A56 was disappeared during the later stages (144–168 h) of fermentation. From Fig. 1c, when comparing the fermentation process with an osmolality-stat control strategy, the acarbose yield increased by 20.42 % under the osmolality-shift fermentation condition, reaching at 4,132.8 ± 111.4 mg/l. Moreover, a significant decline of component C formation was observed, as also shown in Fig. 1c. When using the osmolality-shift

strategy for *Actinoplanes* sp. A56 fermentation, the final accumulation amount of component C was decreased from 498.2 ± 27.1 mg/l to 307.2 ± 9.5 mg/l, with a 38.34 % decline. Further calculations revealed that the final proportion of acarbose/component C was improved from 6.72 to 13.45.

Due to exhibiting inhibitory effects on glycosidase and glucosyltransferase, some researchers attempted to apply C_7-N-aminocyclitols to inhibit the conversion reaction of acarbose to component C. For example, Choi and Shin [3] found that valienamine was a potent inhibitor, and 10 μM of valienamine could reduce component C yields to levels of 38–56 mg/l, corresponding to 89–93 % yield reduction compared with control. Additionally, Xue et al. [20] compared the effects of validamycin A, validamycin B, validamycin D, validamycin E, valienamine, and validamine on acarbose production and component C formation by *Actinoplanes* utahensis ZJB-08196, and the results showed that validamine was the most effective compound, which could make acarbose titer increase from 3,560 ± 128 to 4,950 ± 156 mg/l, and component C concentration concurrently decrease from 289 ± 24 to 107 ± 29 mg/l in batch fermentation after 168 h of cultivation. Although an exogenous addition of C_7-N-aminocyclitols could play a positive role in component C reduction, its high cost would make industrial acarbose fermentation become uneconomical.

During most microbial fermentation processes, a shift strategy of well-direct fermentation parameters, such as pH shift and DO shift, can provoke physiological changes that positively affect process performance, resulting in the improvement of cell growth and metabolites biosynthesis [21]. With broth osmolality as the crucial fermentation parameter, the present work successfully established an osmolality-shift fermentation strategy to improve acarbose production and concurrently reduce component C formation during *Actinoplanes* sp. A56 fermentation process. Although its component C concentration was higher than those obtained under C_7-N-aminocyclitols addition, this kind of osmolality-shift control strategy represented a valuable attraction for industrial acarbose fermentation.

Conclusions

By choosing osmolality level as the key fermentation parameter of acarbose-producing *Actinoplanes* sp. A56, the present work successfully established an effective and simplified osmolality-shift control strategy to improve acarbose production and concurrently reduce component C formation. As a result, the acarbose yield increased by 20.42 %, and the final proportion of acarbose/component C was improved from 6.72 to 13.45.

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