Significantly Enhanced Production of Acarbose in Fed-Batch Fermentation with the Addition of \( S \)-Adenosylmethionine

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Acarbose, a pseudo-oligosaccharide, is widely used clinically in therapies for non-insulin-dependent diabetes. In the present study, \( S \)-adenosylmethionine (SAM) was added to selected media in order to investigate its effect on acarbose fermentation by \( \text{Actinoplanes utahensis} \) ZJB-08196. Acarbose titer was seen to increase markedly when concentrations of SAM were added over a period of time. The effects of glucose and maltose on the production of acarbose were investigated in both batch and fed-batch fermentation. Optimal acarbose production was observed at relatively low glucose levels and high maltose levels. Based on these results, a further fed-batch experiment was designed so as to enhance the production of acarbose. Fed-batch fermentation was carried out at an initial glucose level of 10 g/l and an initial maltose level of 60 g/l. Then, 12 h post inoculation, 100 \( \mu \)mol/l SAM was added. In addition, 8 g/l of glucose was added every 24 h, and 20 g/l of maltose was added at 96 h. By way of this novel feeding strategy, the maximum titer of acarbose achieved was 6,113 mg/l at 192 h. To our knowledge, the production level of acarbose achieved in this study is the highest ever reported.

**Keywords:** Acarbose, \( S \)-adenosylmethionine, fed-batch, \( \text{Actinoplanes utahensis} \)

The \( \alpha \)-glucosidase inhibitor acarbose, a widely available oral drug, has been widely used in the therapy of non-insulin-dependent diabetes mellitus owing to its good therapeutic and non-toxic effects. The drug was first launched in Germany in 1990 and has since been successfully marketed worldwide [5, 23]. Structurally, acarbose is a pseudo-oligosaccharide that consists of the two pseudo disaccharides, acarviosine and maltose. Acarviosine is composed of an unsaturated aminocyclitol connected with a 6-deoxy-\( \beta \)-glucose via an \( N \)-glycosidic bond [2, 14, 24]. The maltose unit in acarbose has been shown to be directly incorporated from maltose or maltotriose, rather than via the successive addition of glucose residues [12].

In recent years, the biosynthetic pathways, and related enzymes and genes for acarbose synthesis have been studied in depth and clearly described [16, 23]. However, major difficulties still exist in improving the yield of acarbose, leading to a high cost for its manufacture. Beunink et al. [1] found that not only low osmolalities (<200 mOsm/kg), but also high osmolalities (>600 mOsm/kg) could cause significantly lower acarbose production, and even completely inhibit its formation [1]. Choi and Shin [4] obtained a better acarbose yield of 3,490 mg/l at 500 mOsm/kg in the presence of 10 \( \mu \)mol/l of valienamine. Moreover, the by-product component C was seen to decrease by 90% to 43 mg/l when compared with the levels obtained in the absence of valienamine. Besides our own previous study [21], until the present time, the maximal acarbose titer in fermentation broth was obtained by Li et al. [13] at 4,327 mg/l in a 30 L fermentor by a fed-batch process, when controlling total sugar and reducing sugar at 75–80 and 45–50 g/l, respectively.

\( S \)-Adenosylmethionine (SAM), a ubiquitous substance in living organisms, is generally known to be a methyl donor in various biosynthetic processes. In previous studies, several researchers have documented that the external addition of SAM, or the overexpression of SAM synthetase, may increase the production of various secondary metabolites in actinomycetes, and possibly functions as a cofactor to provide methyl for methylation reaction, or as a signaling molecule to increase the transcription of pathway-specific regulatory genes [9, 11, 15, 19, 25]. These findings led us to assume that SAM may regulate the synthesis of acarbose.

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in *Actinoplanes*, especially when considering the fact that streptomycin production was enhanced 1.35-fold by the supplementation of SAM at 1 mmol/l [17], and that both acarbose and streptomycin belong to the same family of aminoglycoside antibiotics [6].

We previously reported the isolation and identification of an acarbose-production mutant strain, *A. utahensis* ZJB-08196 [5, 22]. In the present work, we have attempted to determine the effect of the external addition of SAM on the production of acarbose, and excitingly, the results were in accordance with what we had anticipated. Moreover, the effects of glucose and maltose on the production of acarbose, both in batch and fed-batch processes, were investigated in detail. On the basis of these initial experiments, a reasonable strategy for fed-batch fermentation was designed so as to maximize acarbose production. To our knowledge, this is the first report that demonstrates the promise of the small molecule SAM in the regulation of acarbose production.

**Materials and Methods**

**Microorganism, Media, and Cultivation**

The mutant strain of *A. utahensis* ZJB-08196 was used for the production of acarbose in the present study [22]. This strain was kept in a 15% (v/v) glycerol stock solution and stored at −70°C. Before use, *A. utahensis* ZJB-08196 activation was carried out on agar plates at 27°C for about 8 days until visible orange colonies emerged. The medium for the agar plates was as follows (in g/l water): sucrose, 30; peptone, 2; l-Tyr, 1; K$_2$HPO$_4$, 1; KCl, 0.5; MgSO$_4$·7H$_2$O, 0.5; FeSO$_4$·7H$_2$O, 0.1; and the initial pH value was adjusted to 7.0.

For inoculation, a colony of about a 1×1 cm$^2$ size, from a freshly prepared agar plate, was inoculated into a 500-ml shake flask containing 100 ml of seed medium and cultivated at 28°C and 200 rpm for 72 h. The seed medium consisted of (in g/l water) corn starch, 15; soybean flour, 40; glycerol, 20; CaCO$_3$, 2.0; K$_2$HPO$_4$, 0.5; and the initial pH value was adjusted to 6.7 with 6 mol/l NaOH prior to sterilization.

Batch and fed-batch fermentations were carried out by inoculating 10% (v/v) of the seed culture into 500 ml Erlenmeyer flasks containing 50 ml of fermentation medium at 28°C and 200 rpm for 168 h. The basal medium for acarbose fermentation was as follows (in g/l water): maltose, 43; soybean flour, 17; glucose, 40; sodium glutamate, 5; glycerol, 5; FeCl$_3$·6H$_2$O, 0.83; CaCO$_3$, 2.5; K$_2$HPO$_4$, 1; CaCl$_2$, 1.88; and the initial pH value was adjusted to 7.0.

**Exogenous SAM Treatment and Feeding Strategy for the Enhancement of Acarbose Production**

Exogenous SAM treatment was applied to *A. utahensis* ZJB-08196 for the enhancement of acarbose production. Sterile-filtered aqueous solutions of SAM were added into the fermentation medium, and the effects of the added concentrations over time of SAM on acarbose production were investigated. Moreover, the effects of concentrations of glucose and maltose on acarbose production during batch and fed-batch processes were also studied. On the basis of the above experiments, an optimal feeding strategy, coupled with exogenous SAM addition, was performed for maximum acarbose production.

**Analytical Methods**

Samples (5 ml) were centrifuged at 9,000 ×g for 15 min in a pre-weighed tube. The cell precipitates were dried at 80°C to a constant weight for the determination of dry cell weight (DCW) as biomass. The supernatants were used for measuring the other fermentation parameters. Acarbose was analyzed by using a Shimadzu CT8-10AASVP HPLC system by methods previously described [22]. Glucose was estimated with a SBA-40E biosensor (Shandong, China). Maltose was measured according to known methodologies [10].

All experiments were performed three times in duplicate to ensure reliability and accuracy. The data reported herein are the mean ± standard deviation of the results.

**Results and Discussion**

**Impact of Exogenous Addition of SAM on Acarbose Production in Batch Culture**

The exogenous addition of SAM to the culture medium was performed with the final SAM concentration range...