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Full Length Research Paper

Improvement of xanthan gum production in batch culture using stepwise acetic acid stress

Sara Alaei Shehni[1]*, Mohammad Reza Soudi[1], Saman Hosseinkhani[2] and Niloufar Behzadipour[3]

[1]Department of Biology, Faculty of Science, Alzahra University, Tehran, Iran. [2]Department of Biochemistry, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran.
[3]Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, Khuzestan, Iran.

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In this study, the effect of acetic acid on the improvement of xanthan biosynthesis by *Xanthomonas campestris* b82 was investigated. Various concentrations of acetic acid from 1.56 to 6.25 mM were added to the medium in the exponential and stationary phase of growth. Analytical studies revealed a considerable increase in viscosity and xanthan yield after two pulses addition of acetic acid at 24 and 26 h of incubation. ATP assay also showed lower content of ATP in these conditions and was denotative of the consumption of ATP used to produce energy for xanthan biosynthesis. These observations showed the stimulatory effect of acetic acid addition on xanthan production by *X. campestris* b82.

Key words: Xanthan gum, acetic acid, *Xanthomonas campestris* b82, ATP assay.

INTRODUCTION

Xanthan is an extracellular polysaccharide which is mainly produced by the phytopathogenic bacterium *Xanthomonas campestris*, however, other species of *Xanthomonas* such as *Xanthomonas carotae*, *Xanthomonas malvacearum* and *Xanthomonas phaseolis* have been reported to be able to produce the same product (Flores-Candia and Dechwer, 1999; Garcia-Ochoa et al., 2000). It is widely used in food, cosmetic, pharmaceutical and petroleum industries because of its unique rheological properties (Katzbauer, 1998; Rosalam and England, 2006; Flores-Candia and Dechwer, 1999). Although, the global demand for xanthan gum is increasing every year, its cost has remained constant due to parallel increase in production. Various approaches have been considered to improve xanthan productivity, which include improvement of culture condition and medium components, increasing the genetical potency of the strains, and amendments in engineering processes (Funahashi et al., 1988; Nakajima et al., 1990; Marquet et al., 1989; Zaidi et al., 1991; Garcá-Ochoa et al., 1992; Rajeshwari et al., 1995; Jana and Ghosh, 1997; Stredansky and Conti, 1999; Papagianni et al., 2001; Kalogiannis et al., 2003).

In the growth medium for xanthan production, organic acids have been shown to have stimulatory effect on the production of xanthan by *X. campestris* (Peters et al., 1990; Jana and Ghosh, 1997). For example, it has been shown that citric acid that is used as a chelating agent in medium to prevent the precipitation of salts during heat sterilization, can improve xanthan productivity (Jana, 1997).

Acetic acid is a relatively weak carboxylic organic acid which is sometimes used in small amounts in the formation of xanthan gum. Xanthan gum is soluble in this acid; therefore, it provides a good solution to dissolve the xanthan gum. In this study, we indicated acetic acid implication on energetic system of *X. campestris* b82 and xanthan production.

MATERIALS AND METHODS

Microorganism and media

X. campestris strain b82, a native strain previously isolated from soil (Nasrr et al., 2007), was used in this study. The stock culture of the microorganism was maintained on yeast extract agar (YMA) medium containing (g/l): glucose 10.0, yeast extract 3.0, malt

*Corresponding author. E-mail: sara_alaie@yahoo.com. Tel: +98-6113336380 or +98-9166101074.

extract 3.0 and peptone 5.0. The culture was kept at 4°C and renewed every two weeks (Casas et al., 2000; Nasr et al., 2007).

Inoculum preparation

The inoculum was prepared from the broth cultures of *X. campestris* in mid-log phase of growth by transferring the bacteria from the fresh prepared culture of YMA to 10 ml of YM broth medium. After overnight incubation at 28°C with agitation at 150 rpm, 1 ml of the culture was inoculated into 100 ml of fresh YM broth at 28°C to reach OD_{540} of 0.5 to 1. After adjusting the cell concentration at 10^5 to 10^6 CFU/ml using MacFarland 0.5 standard tube, the culture was used to inoculate the batch culture fermentation medium.

Fermentation process

Fermentation was carried out in 500 ml Erlenmeyer flask with 200 ml of a synthetic medium containing (g/l): sucrose 20, $MgSO_4$ .7H2O 0.24 , NH_4Cl 1.2 , Citric acid 2.1 , KH_2PO_4 5 (pH 6.8 to 7.0) without or with various concen-trations of acetic acid (1.56, 3.12 and 6.25 mM). The media were inoculated with a 5% (v/v) of the inoculums followed by incubation in a shaker incubator at 120 rpm and 28 to 30°C. After 72 h, viscosity and xanthan production were evaluated.

Effect of acetic acid

The minimum inhibitory concentration (MIC) of acetic acid was determined. For this assay, two-fold serial dilution of acetic acid was prepared to achieve a dilution range from 1.56 to 400 mM; 1 ml of the acid dilutions were mixed with the equal volume of the synthetic medium; inoculated with *X. campestris b82* followed by 48 h incubation at 28°C. MIC was defined as the lowest acid concentration in which no visible bacterial growth after the incubation period was showed.

The effect of acetic acid on xanthan production was studied with the addition of the un-dissociated form of acetic acid (obtained at pH value close to the acid pKa) in the medium in one pulse, 30 h after incubation (at the end of the exponential growth phase); two pulses, 24 and 26 h after incubation (at the late stage of exponential phase); or three pulses, 22, 24 and 26 h after incubation. The pH of acetic acid reached pKa (4.76) using 6 N NaOH.

Analytical procedures

Fermented cultures were harvested and the produced xanthan was precipitated with isopropanol; collected by filtration and dried at 60°C overnight (El-Salam, 1994). Xanthan yield was determined following the method described previously by Krishna and Sharma, (2000). Viscosity was measured with a brookfield viscometer (Anton Paar DV-1, USA), spindle number 3, and 60 rpm at RT as described by Nitschke and Rodriguse (2000). All the experiments were repeated three times and average values were calculated. 1and 2 h after acetic acid pulses, ATP concentration was assessed by ATP- luciferin-luciferase interaction.

ATP assay

ATP assay was carried out using bioluminescence method which is based on luciferases requirement for ATP in producing light (emission maximum ~560 nm at pH 7.8). Briefly, the cells were harvested and then diluted in ATP-free water to reach approximately 10^6 cells/ml. Serial dilution of ATP in the range of 0.05 to 1.0 mM was used for the preparation of standard curve. ATP was extracted from the cell by intense treatments with sonicator, 3 times with 30 s interval each. After centrifugation at 4000 × *g* for 5 min, the supernatant fractions were used for ATP assay. In a luminometer tube, 10 µl of luciferase reagent and 10 µl of luciferin (5 mM) were mixed with 20 µl of the lysed samples/standards. Luminescence was monitored over a period of 10 s in relative light units (RLUs), 560 nm. The blank (no ATP or no cells) was subtracted from the raw data and ATP concentration was calculated from a log-log plot of the standard curve data.

RESULTS AND DISCUSSION

To study the effect of acetic acid addition in improving xanthan production by *X. campestris* b82, fermentation was carried out in 500-ml shake flasks containing 200 ml of the synthetic medium. To observe the effect of acetic acid, fed batch experiments were conducted in which acetic acid was added to the medium at one (after 24 h), two (after 24 and 26 h) and three pulses (after 22, 24 and 26 h). According to our findings, acid concentrations higher than 6.25 mM (12.5 and 25mM) led to a decrease in the viscosity (data not shown). Therefore, two fold concentrations of acetic acid, from 1.56 to 6.25 mM, in one, two or three pulses was added to the medium and after 72 incubation at 28-30°C, rheological properties of xanthan was assessed. As shown in Figure 1, at the end of fermentation in the medium with one pulse addition of acetic acid in the early stage of stationary phase (30 h after starting the fermentation process), an increase in xanthan viscosity was observed. However, rheological properties of xanthan after 2 pulses addition, especially at the concen-tration of 6.25 mM showed a considerable improvement when compared with the control. No significant improve-ment was observed after 3 pulses addition of the acid.

Increase in xanthan concentration was observed to depend on the concentrations and pulse numbers of acetic acid addition (Table 1). Consistent with the viscosity results, xanthan formation was observed to be improved when acetic acid feeding was carried out in two pulses of 6.25 mM after 24 and 26 h of incubation and more addition did not have any remarkable effects on xanthan synthesis.

Xanthan synthesis requires energy produced by consuming ATP by *X. campestris* (Pons et al., 1989). During xanthan production, considering the ratio of acetate to pyruvate as 0.60:0.38, 10.89 mol of ATP is consumed and 3.58 mol of NADH + H^+ is produced (Pons et al., 1989; Jana and Ghosh, 1997; Vashitz and Sheintuch, 1991). Our data on the ATP assay showed that the ATP content of the bacterial cells decreased after pulse addition of acetic acid in the exponential and stationary phases of growth. As shown in Figure 2, after the first (22 h) and second pulses (24 h) of acid addition, ATP content decreased, this reduction was more in the presence of 6.25 mM of acetic acid. After the third addition, no significant decrease in ATP concentration

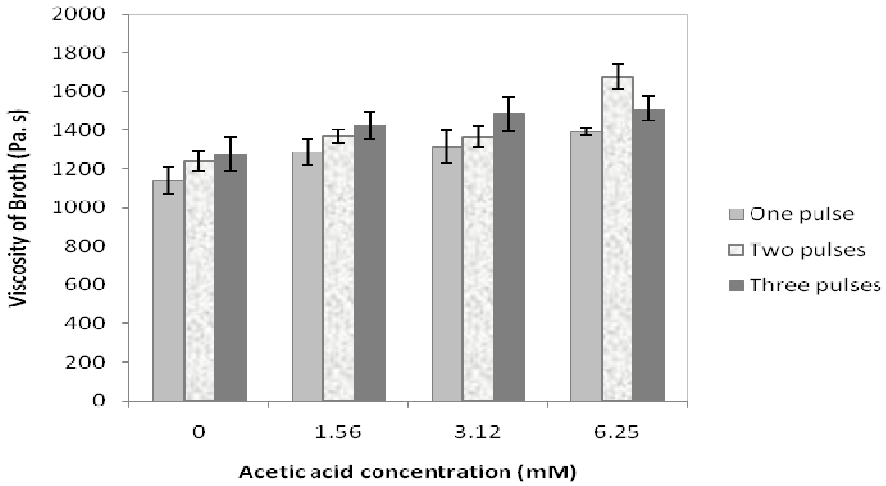

Figure 1. Effect of acetic acid addition on the viscosity of the broth culture of *X. campestris*. Viscosity of the broth medium was measured in the presence of various concentrations of acetic acid that were added to the medium in 1, 2 or 3 pulses. A significant increase in viscosity was observed after adding 6.25 mM of acid in 2 pulses after 24 and 26 h of fermentation, respectively. Bars represent averages ± SD (n = 3).

Table 1. Effect of acetic acid addition on xanthan production by *X. compestris*.

Concentration of acetic acid (mM)	Xanthan yield[a] (g/l) in the presence of acetic acid		
	After the first pulse	After the second pulse	After the third pulse
0	8.85±0.21	8.45±0.35	8.85±0.21
1.56	9.50±0.56	9.30±0.42	9.55±0.78
3.12	9.38±0.22	9.86±0.23	9.70±1.70
6.25	10.25±0.61	11.50±0.50	10.05±0.17

[a]Xanthan weight after biomass removal.

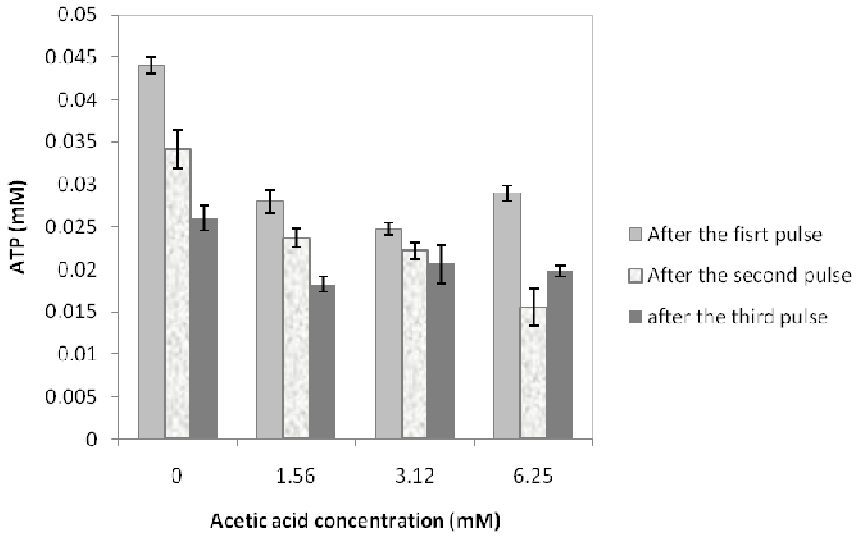

Figure 2. Measurement of ATP content in *X. campestris* in the presence of acetic acid. Bacterial cells were cultured in the synthetic medium with various concentrations of acetic acid; ATP was extracted from the cells by sonication and then measured by luciferase assay. Bars represent averages ± SD (n = 3).

was observed. This may be due to bacterial adaptation and acidic condition. Low concentration of ATP in the treated cells with acetic acid, with a significant increase in xanthan production showed the consumption of ATP for xanthan synthesis.

In summary, the results of this study show the positive effect of acetic acid addition on biosynthesis of xanthan by *X. campestris b82*.

REFERENCES

Casas J, Santos V, García-Ochoa F (2000). Xanthan gum production under several operational conditions molecular structure and rheological properties. Enzymes Microbial. Technol. 26: 282-291.

El-Salam A (1994). Bioconversion of sugarcane molasses into xanthan gum. J. Biotechnol. 33(1): 103-106.

Flores-Candia J, Dechwer W (1999). Xanthan gum. In Flickinger M & Derw S (Eds.), In Encyclopedia of Bioprocess. Technol. Fermentation, Biocatalysis. Bioseparation. New York Wiley and Sons Inc. 5: 2695-3711.

Funahashi H, Hirai K, Yoshida T, Taguchi H (1988). Mixing state of xanthan gum solution in an aerated and agitated fermentor-Effects of impeller size on volumes of mixing regions and circulation time distribution. J. Ferment. Technol. 66(1): 103-109.

Garcá-Ochoa F, Santos V, Fritsch A (1992). Nutritional study of *Xanthomonas campestris* in xanthan gum production by factorial design of experiments. Enzyme Microbial. Technol. 14(12): 991-996.

Garcia-Ochoa F, Santos V, Casas J, Gomez E (2000). Xanthan gum production, recovery, and properties. Biotechnol. Adv. 18(7): 549-579.

Jana A, Ghosh P (1997). Stimulation of xanthan production by *Xanthomonas campestris* using citric acid. World J. Microbiol. Biotechnol. 13(3): 261-264.

Kalogiannis S, Iakovidou G, Liakopoulou-Kyriakides M, Kyriakidis D, Skaracis G (2003). Optimization of xanthan gum production by *Xanthomonas campestris* grown in molasses. Process Biochem. 39(2): 249-256.

Katzbauer B (1998). Properties and applications of xanthan gum. Polym. Degra. Stabil. 59: 81-84.

Krishna LJ, Sharma G (2000). Studies on xanthan production from *Xanthomonas campestris*. Bioproc. Biosyst. Eng. 23(6): 687-689.

Marquet M, Mikolajczak M, Thorne L, Pollock T (1989). Improved strains for production of xanthan gum by fermentation of *Xanthomonas campestris*. J. Ind. Microbiol. Biotechnol. 4(1): 55-64.

Nakajima S, Funahashi H, Yoshida T (1990). Xanthan gum production in a fermentor with twin impellers. J. Ferment. Bioeng. 70(6): 392-397.

Nasr S, Soudi M, Haghighi M (2007). Xanthan production by a native strain of *X. campestris* and evaluation of application in EOR. Pak. J. Biol. Sci. 10: 3010-3013.

Nitschke M, Rodrigues V (2000). Effect of virulence and serial transfers of *Xanthomonas campestris* on xanthan gum production. Braz. J. Microbiol. 31(1): 58-60.

Papagianni M, Psomas S, Batsilas L, Paras S, Kyriakidis D, Liakopoulou-Kyriakides M (2001). Xanthan production by Xanthomonas campestris in batch Cult. Process Biochem. 37(1): 73-80.

Peters H, Ghosh P, Zaidi A, Schumpe A, Deckwer W (1990). *The continuous production of xanthan gum.* Paper presented at the Fifth European Congress on Biotechnol. Copenhagen.

Pons A, Dussap C, Gros J (1989). Modelling *Xanthomonas campestris* batch fermentations in a bubble column. Biotechnol. Bioeng. 33(4): 394-405.

Rajeshwari K, Prakash G, Ghosh P (1995). Improved process for xanthan production using modified media and intermittent feeding strategy. Lett. Appl. Microbiol. 21(3): 173-175.

Rosalam S, England R (2006). Review of xanthan gum production from unmodified starches by *Xanthomonas comprestris* sp. Enzyme. Microbial. Technol. 39(2): 197-207.

Stredansky M, Conti E (1999). Xanthan production by solid state fermentation. Process Biochem. 34(6-7): 581-587.

Vashitz O, Sheintuch M (1991). Analysis of polymer synthesis rates during steady state growth of *X. campestris*. Biotechnol. Bioeng. 37(4): 383-385.

Zaidi A, Ghosh P, Schumpe A, Deckwer W (1991). Xanthan production in a plunging jet reactor. Appl. Microbiol. Biotechnol. 35(3): 330-333.