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Short communication

Effect of Tween 80 on the production of curdlan by *Alcaligenes faecalis* ATCC 31749

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ABSTRACT

This study aims to investigate the effects of Tween 80 on curdlan production, cell growth, and glucosyltransferase activity. The addition of Tween 80 to the culture medium increased curdlan production. However, curdlan production did not increase further when excessive Tween 80 (>0.3% Tween 80) was added to the culture medium. The addition of Tween 80 to the culture medium did not affect cell growth. The glucosyltransferase activity involved in the curdlan synthesis increased with the increase of Tween 80 concentration. The glucosyltransferase activity did not increase further when excessive Tween 80 (>0.3% Tween 80) was added to the culture medium. Maximum curdlan was observed at day 5 and then levelled off. The biomass continued to increase until the end of the experimental period (6 d). Maximum glucosyltransferase activity was also observed at day 5 and decreased thereafter. The results indicate that the enhanced curdlan production by Tween 80 is highly correlated with glucosyltransferase activity.

1. Introduction

Exopolysaccharide curdlan, a linear glucan interconnected by β-(1→3) glucosidic linkages, is produced by the *Agrobacterium* species or *Alcaligenes faecalis* (Harada, Fujimori, Hirose, & Masada, 1966). This biopolymer is a thickener with unique thermal gelling properties and is widely used in the food industry. Modified curdlan has pharmaceutical applications such as controlled drug delivery (Jiang, 2013).

Attempts have been made to enhance curdlan production to meet the great demand in such applications. A high concentration of curdlan can be obtained by optimising various crucial factors, including large-scale submerged fermentation, carbon source, nitrogen source, phosphate concentration, pH, and agitation speed (Jiang, 2013; Kim et al., 2000; Lee & Park, 2001; Lee et al., 1999; Salah et al., 2011; Wang, Zhan, Zhu, Li, & Yang, 2002).

The mechanism of exopolysaccharide biosynthesis has been investigated through a number of studies in recent years (Daran, Dallies, Thines-Sempoux, Paquet, & Francois, 1995; Duan, Chi, Wang, & Wang, 2008; Pan, Yao, Chen, & Wu, 2013). Duan et al. observed that cells have higher glycosyltransferase activities when more curdlan is synthesised (2008).

Tween 80, a non-ionic surfactant, has been reported to enhance the glucosyltransferase activity in *Streptococcus mutants* (Tomita et al., 1998) and *Leuconostoc dextranicum* (Majumder & Goyal, 2008). However, the relationships among Tween 80 concentration, the glucosyltransferase activity, and curdlan production remain undetermined.

The specific objective of this paper is to investigate the effects of Tween 80 on the curdlan production, biomass accumulation, and the glucosyltransferase activity of *A. faecalis* ATCC 31749.

2. Materials and methods

2.1. Microorganism

A. faecalis ATCC 31749 was used. The stock cultures were maintained on potato dextrose agar at 4 °C and were subcultured every two weeks.

2.2. Preparation of inoculum medium fermentation medium and fermentation medium

The inoculum medium contained glucose (20 g L^{-1}), yeast extract (10 g L^{-1}), KH_2PO_4 (1.74 g L^{-1}) and MgSO4·$7H_2O$ (0.49 g L^{-1}). The fermentation medium contained glucose (50 g L^{-1}), urea (0.30 g N L^{-1}), KH_2PO_4 (2.0 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), $FeCl_3$ (1.0 g L^{-1}), $MnCl_2$ (1.0 g L^{-1}), $CaCl_2$ (1.0 g L^{-1}), Tween 80, and NaCl (1.0 g L^{-1}) (Jiang, 2013). The media were autoclaved at 121 °C for 15 min.

2.3. Fermentation

The seed cultures were incubated in 250 mL shake flasks at 30 °C and 200 rpm for 24 h and 5 mL of this seed cultures was transferred

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into 500 mL flasks containing 100 mL of the fermentation media. The cultures were shaken at 200 rpm at 30 °C for 6 d (Jiang, 2013).

2.4. Curdlan and biomass determination

Curdlan production and biomass were determined by the gravimetric method described by Yu et al. (2011). The fermentation broth was centrifuged at 8000 × *g* for 30 min at 4 °C. The pellets containing cells and curdlan were resuspended in NaOH solution (0.5 mol L^{-1}) for 1 h. The cells were separated by centrifugation at 8000 × *g* for 30 min. The resulting curdlan in the supernatant was precipitated by adding 2.0 mol L^{-1} HCl. The cells and curdlan were washed and dried to a constant weight in an oven at 80 °C.

2.5. Enzyme assays

The glucosyltransferase activity was determined according to the methods described by Duan et al. (2008). A suitably diluted enzyme preparation (0.2 mL) was incubated with pre-equilibrated *p*-nitrophenyl α-D-glucopyranoside (0.2 mL, 10 mM) in 0.1 M sodium acetate buffer (pH 4.0) at 40 °C for 5 min. The reaction was terminated by adding an aqueous solution of Trizma base (3.0 mL, 2.0%, w/v). The absorbance at 410 nm was measured. The glucosyltransferase activity was expressed in terms of μM of *p*-nitrophenol released/min and was calculated based on a standard curve (the standard curve was obtained by measuring $OD_{410\,nm}$ values of the solutions with different concentrations of *p*-nitrophenol). The mixture with the diluted enzyme preparation that had been heated at 100 °C for 5 min was used as the control.

2.6. Data analysis

The SPSS 13.0 programme was used to analyse the standard deviation and significance of the data. The statistical significance was set to $p < 0.05$.

3. Results and discussion

3.1. Effect of Tween 80 concentration on curdlan production, cell growth and the glucosyltransferase activity

The effects of varying concentrations of Tween 80 on curdlan production and cell growth by *A. faecalis* ATCC 31749 is shown in Fig. 1. The maximum curdlan production (21.22 g L^{-1}) and the highest glucosyltransferase activity (3.91 U/mg of protein) were observed in a medium containing 0.3% Tween 80. Tween 80 is an excellent surfactant and can increase the permeability of the lipid bilayer membrane of the cell. Therefore, Tween 80 could be favourable in curdlan secretion. Tween 80 in the media could also increase the homogeneity of the broth and facilitate the transfer of the nutrient to the cell. However, curdlan production and the glucosyltransferase activity did not increase further when Tween 80 concentration exceeded 0.3%. This result could be ascribed to the fact that excessive Tween 80 concentrations could not decrease the surface tension of the broth and could not increase the permeability of the lipid bilayer membrane of the cell and the homogeneity of the broth. The presence of Tween 80 in the nutrient medium did not affect cell growth, indicating that Tween 80 is not a nutrient for *A. faecalis* ATCC 31749.

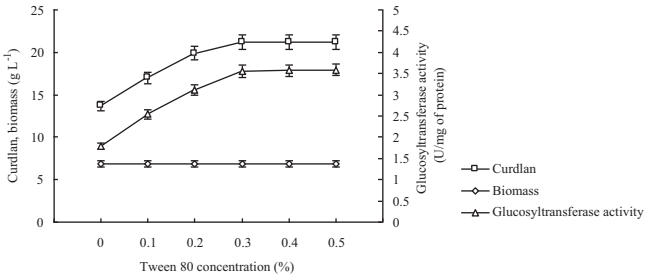

Fig. 1. Effect of Tween 80 concentration on curdlan production, cell growth, and glucosyltransferase activity. Data are shown as mean ± SD (*n* = 3).

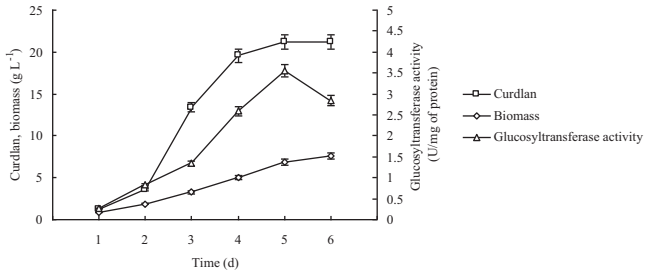

Fig. 2. Effect of time course on curdlan production, cell growth, and glucosyltransferase activity. Data are shown as mean ± SD (*n* = 3).

3.2. Kinetics of fermentation

Fermentation by *A. faecalis* ATCC 31749 was conducted for a period of 6 d. Curdlan production increased sharply from day 2 to day 4, increased slightly from day 4 to day 5 and did not increase after day 5 (Fig. 2). The biomass steadily increased over the entire fermentation period (Fig. 2). The results show that curdlan production is not associated with cell growth. The glucosyltransferase activity peaked at day 5 and decreased sharply after day 5 (Fig. 1). Therefore, the glucosyltransferase activity was highly correlated with curdlan biosynthesis. The optimum fermentation time is similar to that observed by Jiang (2013).

4. Conclusions

The effects of the addition of Tween 80 to the fermentation medium on the curdlan production, cell growth, and the glucosyltransferase activity of the *A. faecalis* ATCC 31749 culture were investigated. The maximum curdlan production and glucosyltransferase activity were obtained at day 5 with 0.3% Tween 80 in the medium. The biomass continued to increase until the end of the experimental period (6 d). The glucosyltransferase activity was highly correlated with curdlan production.

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