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A N I N T E R N A T I O N A L J O U R N A L

Optimization of Fermentation Medium for Enhanced Glucansucrase and Glucan Production from *Weissella confusa*

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ABSTRACT

A glucan producing Weissella confusa *isolated from fermented cabbage, as identified earlier, was used for optimization of its fermentation medium. The effects of various macronutrients such as sucrose, glucose as cosubstrate, yeast extract, beef extract, peptone, sodium acetate, K_2HPO_4 and Tween 80 were studied on glucansucrase and glucan production from* Weissella confusa*. The medium used as control gave 6.0 U/ml enzyme activity and 34 mg/ml glucan concentration. Sucrose (5%), glucose as cosubstrate (5% for glucansucrase and 3% for glucan production), Tween 80 (0.1%), yeast extract (1.5%), Peptone (2.0%) and K_2HPO_4 (1.5%) were effective nutrients displaying higher glucansucrase and glucan production giving 18.2, 18.0, 7.0, 6.4, 6.2 and 6.4 U/ml enzyme activity and 103, 100, 46, 41, 39 and 37 mg/ml glucan concentration, respectively. Sodium acetate and beef extract were not effective for enzyme and glucan production. The new strain* Weissella confusa *can be used for commercial production of glucansucrase and glucan using optimized medium.*

Key words: *Weissella confusa*, glucansucrase, glucan, Tween 80

INTRODUCTION

Glucans are class of polysaccharides of varing structure with contiguous α(1→6) glycosidic linkages in the main chain and varing percentage of α(1→2), α(1→3), and α(1→4) linkages. The enzyme responsible for glucan formation is known as glucansucrase and are produced by lactic acid bacteria belonging to the genera: *Leuconostoc, Lactobacillus and Streptococcus* and belongs to family 70 of glycoside hydrolases (Henrissat and Davies, 1997). Recently *Weissella sp.* has drawn attention for its high glucan production capacity which is more linear in nature (Maina et al., 2008) as compared to 95% linearity in glucan formed from *Leuconostoc mesenteroides* NRRL B512F (Seymour and Knap, 1980). Glucansucrases are high molecular weight, inducible or constitutive enzyme which catalyze the polymerization of the glucopyranosyl moieties of sucrose to form glucan. The enzyme glucansucrase remains in an aggregated form in the presence of glucan resulting in a high molecular weight (Funane et al., 1995).

There are several reports on sucrose effect on glucansucrase production by various strains of *Leuconostoc* spp. For higher dextran production high sucrose concentration in the medium has been used (Hehre, 1946). Tsuchiya et al., 1952 studied the effect of sucrose, corn steep liquor and

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phosphate on dextransucrase production. Goyal and Katiyar, 1997 used low yeast extract and high K_2HPO_4 concentration for enhanced enzyme yield. However, glucansucrase production by wild-type *L. mesenteroides* grown on glucose or maltose instead of sucrose also has been reported (Smith and Zahnley, 1999). Behravan et al., (2003) used sugar-beet molasses as a sucrose source and wheat bran as substitute for yeast extract. The *L. mesenteroides* NRRL B-512F displayed increased production of biomass and enzyme with the increase in K_2HPO_4 concentrations (Rodrigues et al., 2003). There are few reports on the use of glucose as cosubstrate for glucansucrase and glucan production. Dols et al., 1996 reported effect of added glucose, fructose and mannitol on dextransucrase and dextran yield from *Leuconostoc mesenteroides* NRRL B-1299. The aeration conditions were optimized for production and scale up of dextransucrase from *Leuconostoc mesenteroides* in a bioreactor (Michelena et al., 2003).

In our earlier study high glucan production ability of *Weissella confusa* (Cab3) isolated from fermented cabbage was explored and compared with other lactic acid bacteria (Shukla and Goyal, 2011). Glucan production has typically served as a phenotypic test in the identification of bacteria classified in the genus *Weissella* (Collins et al., 1993). *Leuconostoc mesenteroides* NRRL B-512F used industrially also produces levansucrase in lower amounts along with glucansucrase (Robyt and Walseth, 1979). Whereas *Weissella confusa* elaborates only glucansucrase and there is no contamination of levansucrase (Shukla and Goyal, 2011). It is a prerequisite to formulate a medium for fermentation process to obtain maximum biomass and high yields of desired metabolic products. So, there is a requirement of the optimization of glucansucrase and glucan production from *Weissella confusa* which is also a hyper glucan producer. To best of our knowledge there are only a few reports on the production of glucansucrase and glucan from *Weissella* species (Maina et al., 2008, Katina et al., 2009). This novel strain produces 6.0 U/ml glucansucrase and 34 mg/ml glucan in the enzyme production medium (Shukla and Goyal, 2011), without any medium or culture condition optimization. The values are quite high as compared to glucansucrase and glucan production from other strains. In the present study the effects of carbon sources, nitrogen sources and buffering agent on glucansucrase and glucan production from *Weissella confusa* were studied.

MATERIAL AND METHODS

Microorganisms and its maintenance

The strain *Weissella confusa* (Cab3) (GenBank Accession Number: GU138518.1) (Shukla and Goyal, 2011) was isolated from fermented cabbage. The culture was maintained in modified MRS (sucrose replaced by glucose) (Goyal and Katiyar, 1996), as stab at 4ºC and sub-cultured every 2 weeks. For the production of glucansucrase and glucan a loop of culture from an agar stab was transferred to 5 ml of sterile medium described by Tsuchiya et al., 1952. The cultures were grown at 25°C with 180 rpm for 12-16h. 1% of the culture inoculum was used for the enzyme production from *Weissella confusa* (Shukla and Goyal, 2011).

Production of glucansucrase and glucan

All the fermentations were carried out using enzyme production medium described by Tsuchiya et al. (1952) that contained (%, w/v) sucrose, 2; yeast extract, 2; K_2HPO_4, 2; $MgSO_4.7H_2O$, 0.02; $MnSO_4.4H_2O$, 0.001; $FeSO_4.7H_2O$, 0.001; $CaCl_2.2H_2O$, 0.001; NaCl, 0.001 and the pH was adjusted to 6.9. All fermentations were carried out in triplicate sets of 60 ml enzyme production medium (EPM) in 250 ml Erlenmeyer flask incubated at 25°C under shaking condition at 180 rpm. The samples (1 ml) were withdrawn at indicated time intervals and centrifuged at 8,000*g* for 10 min at 4°C to separate the cells. The cell free supernatant was analyzed for enzyme activity protein concentration and glucan content.

Glucansucrase activity assay

The enzyme assay was carried out in 1 ml reaction mixture containing 5% (w/v) sucrose, 20 mM sodium acetate buffer (pH 5.4) and 20 µl cell free supernatant. The enzymatic reaction was performed at 30ºC for 15 min. 100 µl aliquot from the reaction mixture was taken for reducing sugar estimation. The enzyme activity was determined by estimating the released reducing sugar by Nelson (1944) and Somogyi (1945) method. The absorbance of the colour developed was measured by spectrophotometer at 500 nm. Fructose was used to plot the standard graph.

Estimation of glucan

The polysaccharide content of the isolated *Weissella confusa* strain (Cab3) was determined by phenol-sulphuric acid method (Dubois, 1956) in a micro-titre plate (Fox and Robyt 1991). The isolated strain was grown in 60 ml liquid medium described by Tsuchiya (Tsuchiya et al., 1952) at 25^{o}C and 180 rpm upto 32 h. The samples (1 ml) were withdrawn at regular intervals. To 200 µl of the culture supernatant three volume of the pre-chilled ethanol was added and centrifuged at 12,000*g*. The supernatant was discarded and the precipitate was resuspended in 200 µl distilled water. The process was repeated two more times. The final precipitate was air dried and dissolved in 200 µl distilled water. Glucan T-40 was used to plot standard.

Effect of sucrose on glucansucrase and glucan production

The effect of sucrose concentration on glucansucrase production was studied by varying its concentration from 1 to 6% in the enzyme production medium by keeping the concentration of other components constant. The medium containing the 2% sucrose was considered as control.

Effect of glucose as cosubstrate on glucan-sucrase and glucan production

To study the effect of glucose as cosubstrate on glucansucrase and glucan production, glucose was supplemented in the enzyme production medium along with sucrose. The concentration of glucose was varied from 1 to 5% by keeping the sucrose concentration, 2% in each case. The concentrations of other components were kept constant. The medium containing the 2% sucrose and 0% glucose was considered as control.

Effect of nitrogen sources on glucansucrase and glucan production

Three different nitrogen sources *viz.* yeast extract, beef extract and peptone were investigated for their effects on glucansucrase and glucan production. The concentrations of nitrogen sources were varied from 1% to 4%. The concentrations of other components were kept constant.

Effect of sodium acetate, Tween 80 and K_2HPO_4 on glucansucrase and glucan production

Sodium acetate concentration was varied from 0.05 to 0.3% to observe its effect on enzyme and glucan production. Medium without sodium acetate was considered as control. The effect of Tween 80 on enzyme and glucan production was studied by varying its concentration from 0.02 to 0.3% (v/v) in the medium. The medium without Tween 80 served as a control. The 1 to 3% K_2HPO_4 was used to study its effect on enzyme and glucan production. The enzyme production medium (Tsuchiya et al., 1952) containing 2% K_2HPO_4 was considered as control.

RESULTS AND DISCUSSION

Effect of sucrose on glucansucrase and glucan production

Among the nutrients, carbon source sucrose was chosen as it induces the glucansucrase production and is also, a substrate for glucan production from *Weissella confusa* (Cab3). The increase in glucansucrase activity and glucan concentration was 3-fold, from 6.0 U/ml to 18.2 U/ml and from 34 mg/ml to 102 mg/ml (Fig. 1, Table 1), with an increase in sucrose concentration from 2% (control) to 5%, in the medium.

As the sucrose concentration increased there was an increase in viscosity of the broth due to the subsequent formation of exopolysaccharide from the available sucrose by the released enzyme, in the medium. Above 5% sucrose concentration the enzyme activity and glucan concentration started decreasing. This could be due to some inhibitory effect of substrate on enzyme production as the substrate concentration is increased beyond 5%. Increment in sucrose concentration is also known to enhance the enzyme activity in other lactic acid strains *viz. Leuconostoc mesenteroides* NRRL B 512F where increase in sucrose concentration from 2% to 4%, a 1.7 times increase in the glucansucrase activity was observed (Goyal and Katiyar, 1997). *Leuconostoc mesenteroides* NRRL-B640 also showed 3 fold increase in the glucansucrase activity from 4.8 to 15 U/ml, with an increase in sucrose concentration from 2 to 7% (Purama and Goyal, 2008).

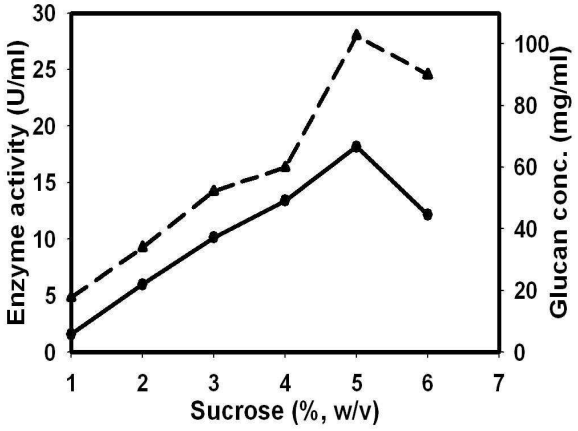

Figure1 - Effect of sucrose on glucansucrase (—●—) and glucan (---▲---) production.

Table 1 - Effect of nutrients on enzyme activity and glucan concentration.

Medium component	Enzyme activity (U/ml)	Glucan conc. (mg/ml)
Control medium*	6.0 (±0.2)	34
Sucrose (5%)	18.2	103
Glucose as cosubstrate	18.0 (5%)	100 (3%)
Yeast extract (1.5%)	6.4	41
Peptone (2%)	6.2	39
Beef extract (3%)	4.8	25
Sodium acetate (0.05%)	6.0	34
Tween 80 (0.1%)	7.0	46
K_2HPO_4 (1.5%)	6.4	37

Effect of glucose as cosubstrate on glucan-sucrase and glucan production

The sucrose is essentially required for the induction of glucansucrase. The effect of glucose as cosubstrate with sucrose on glucansucrase and glucan production was studied. Low initial glucose concentration (1% to 2%), had no effect on enzyme production, but as the glucose concentration increased beyond 2%, the enzyme production started increasing (Fig. 2). The enzyme activity increased by 3.3 fold (5.5 U/ml to 18.0 U/ml) as the glucose concentration was increased from 1% to 5%.

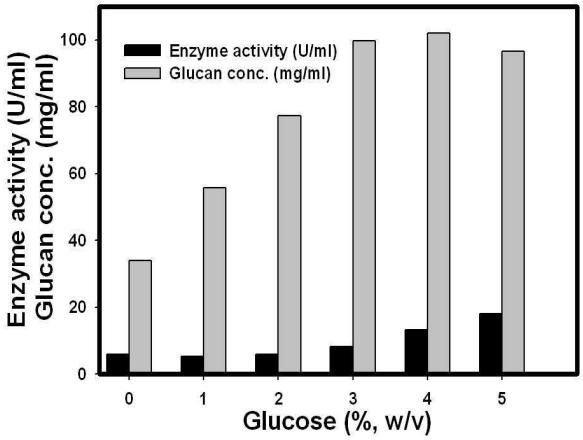

Figure 2 - Effect of glucose as cosubstrate on glucan-sucrase and glucan production.

This is in contrast to the results obtained by Dols et al., 1996 where the relative enzyme activity decreased by 6% as compared to control medium, as the glucose (1.2%) as cosubstrate was included in the medium. Addition of glucose in the medium also significantly increased the glucan production. Glucan concentration in the standard medium was 34 mg/ml which on addition of glucose (3%) significantly increased 3 fold to 100 mg/ml (Fig. 2, Table 1). Beyond 3% glucose there was only marginal increase in the glucan production. Dols et al., 1996 also reported similar results where addition of glucose as cosubstrate in the medium increased the glucan yield by 27% as compared to control medium.

Effect of nitrogen sources on glucansucrase and glucan production

Effect of various nitrogen sources like yeast extract, peptone and beef extract on glucansucrase and glucan production by *Weissella confusa* were studied. Yeast extract was most effective nitrogen source for glucansucrase and glucan production. At 1.5% (w/v) concentration, 6.4 U/ml enzyme activity and 41 mg/ml glucan concentration were observed (Fig. 3A, Table 1), which is 7% and 21% more than that observed in control medium containing 2% yeast extract. As the concentration increased beyond 2%, there was no change in enzyme activity. However, higher concentration of yeast extract inhibited glucan production. Peptone significantly increased glucansucrase and glucan concentration from 2.3 U/ml to 6.2 U/ml and 11 mg/ml to 39 mg/ml respectively, as the concentration of peptone was increased from 1 to 2%. Beyond 2% peptone also started inhibiting glucan production (Fig. 3B) as observed with yeast extract. Beef extract neither supported glucansucrase activity nor glucan production. Increasing beef extract concentration from 1-3%, increased glucansucrase and glucan production from 2.5 to 4.8 U/ml and 13 to 25 mg/ml, respectively (Fig. 3C). Both the values of maximum enzyme activity (4.8 U/ml) and glucan concentration (25 mg/ml) are much lower as compared to that of control medium containing 2% yeast extract (Table 1). From the results it was concluded that higher concentrations of nitrogen sources inhibited glucansucrase or glucan production which could be due to their complex nature.

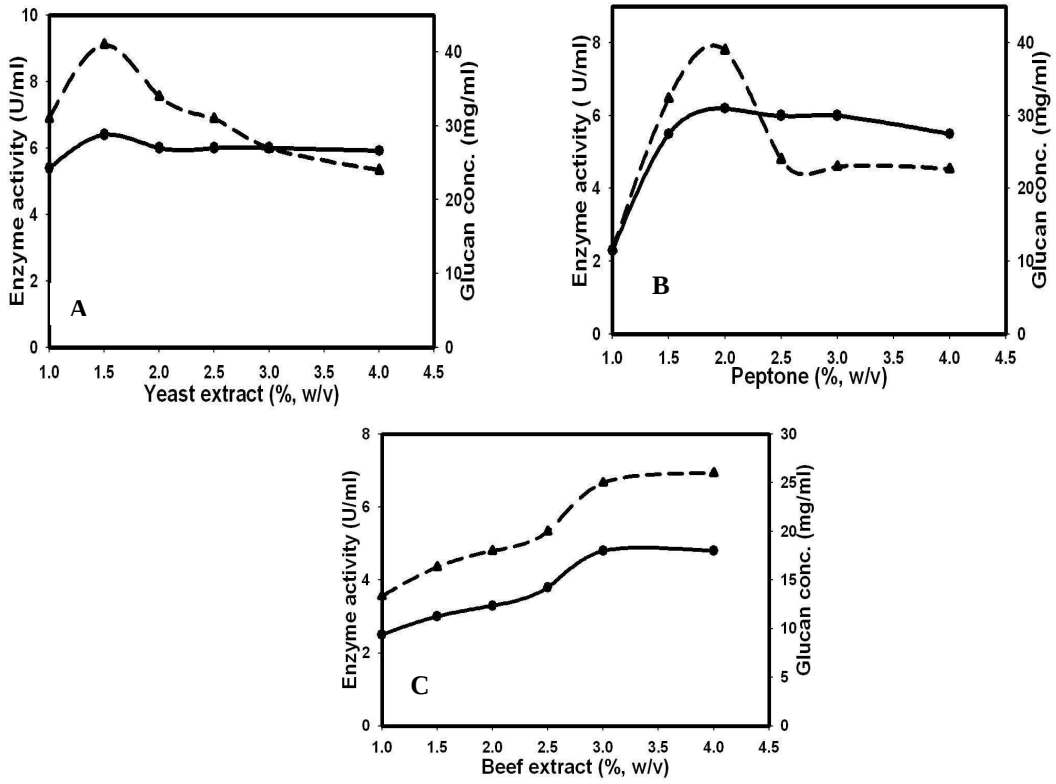

Figure 3 - Effect of (A) Yeast extract (B) Peptone and (C) Beef extract on glucansucrase (—●—) and glucan (---▲---) production.

Effect of Tween 80, sodium acetate and K_2HPO_4 on glucansucrase and glucan production

Sodium acetate acts as a buffering agent and helps to maintain the pH in the fermentation process (Kim et al., 2003). Sodium acetate was used in the medium to study its effect on glucansucrase and glucan production. At lower concentration (0.05%, w/v), it did not have any effect on glucansucrase as well as glucan production (Fig. 4, Table 1). However, beyond 0.05% concentration sodium acetate negatively affected both. Whereas, Sawale and Lele (2010) reported positive effect of sodium acetate (1.51% w/v) on dextransucrase and dextran production by a *Leuconostoc mesenteroides* strain isolated from fermented idli batter.

The production of glucansucrase and glucan increased with increase in concentration of Tween 80 upto 0.1% (v/v) (Fig. 5). This result is similar to the earlier reports where they showed that addition of Tween 80 to enzyme production medium altered the fatty acid composition of the membrane thus enhancing the secretion of the dextransucrase and its activity (Umesaki et al., 1977; Sato et al., 1989; Goyal and Katiyar, 1997; Purama and Goyal, 2008). In present study 0.1% Tween 80 gave a 17% and 35% increase in enzyme activity and glucan concentration respectively. Further increase in the Tween 80 concentration showed saturation in glucansucrase and glucan production full stop 1.5% (w/v) K_2HPO_4 concentration was optimum for both glucansucrase as well as glucan production which gave 6.4 U/ml and 37 mg/ml enzyme activity and glucan concentration respectively (Fig. 6, Table 1). Beyond 1.5% (w/v) K_2HPO_4, both glucansucrase and glucan production decreased.

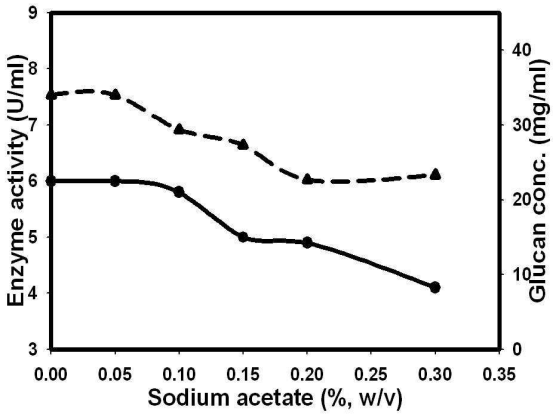

Figure 4 - Effect of Sodium acetate on glucansucrase (——●——) and glucan (---▲---) production.

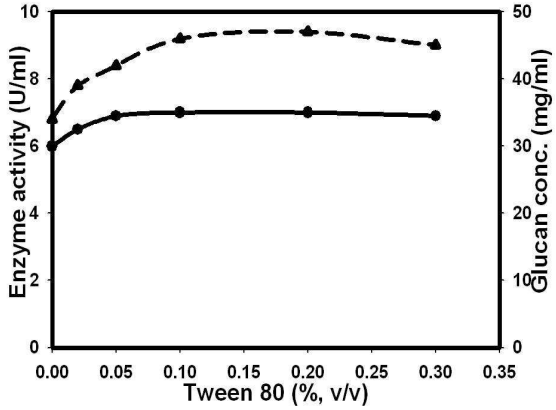

Figure 5 - Effect of Tween 80 on glucansucrase (——●——) and glucan (---▲---) production.

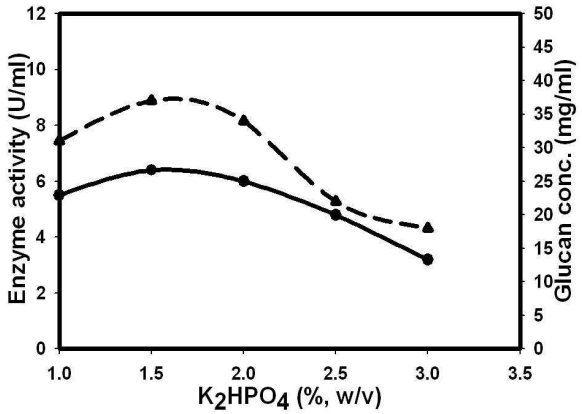

Figure 6 - Effect of K_2HPO_4 on glucansucrase (——●——) and glucan (---▲---) production.

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