Production of Commercially Important Glucansucrase from a Newly Isolated Strain of Leuconostoc mesenteroides AA1
A Aman, S Ul Qader, S BAno, S Iqbal, A Azhar

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Abstract
Glucansucrase is an industrially important extracellular enzyme produced by Leuconostoc mesenteroides. This glucansucrase is widely used for glucan production. Both the product and the enzyme have received increased attention because of their wide range of applications. Due to this, isolation of new strains of L. mesenteroides for glucansucrase production is of great interest. In this work eleven different strains of Leuconostoc mesenteroides were screened and isolated from locally available fruits and vegetables. A new glucansucrase producing strain of Leuconostoc mesenteroides AA1 have been isolated from Brassica oleracea var Capitata L. and was selected on the basis of high enzyme productivity. Various experimental conditions and aspects regarding glucansucrase production were studied. This newly isolated strain optimally produces maximum glucansucrase enzyme after 8 hours of incubation in a new medium designed for enzyme production. Maximum glucansucrase was produced at a sucrose concentration of 2.5% when incubated at 25°C and pH 7.5. Under these conditions 53.0DSU/ml/hr units were produced. This new strain Leuconostoc mesenteroides AA1 could be used for the commercial production of glucansucrase and can also facilitate glucan production on large scale.

INTRODUCTION
Glucansucrase [2.4.1.5] commonly known as dextransucrase is a glucosyltransferase, that is responsible for glucan (dextran) synthesis from sucrose and it catalyzes the transfer of glucosyl residue from sucrose to the growing glucan polymer, liberating fructose as a byproduct (Sidebotham, 1974).

Glucansucrase and glucan have applications in various industries like pharmaceutical, food, cosmetic, agricultural, as well as in photography and mining. Specific molecular weight fractions of glucans are used in medicine, flocculation, stabilization, lyophilization, protective colloids in blood-expanders and cosmetic ingredients formulation (Kim & Day, 1994; Leather et al. 1995; Shamala & Prasad, 1995; Sutherland et al. 1996). Crosslinked glucans are used as gel permeation matrices in research and industries for separation purposes of various products (Alsop, 1983).

Several species of genera Leuconostoc, Lactobacillus and Streptococcus have been found to synthesize glucansucrase. Its expression is constitutive in Streptococcus strains (Ciardi et al. 1997), while it is inducible in Leuconostoc strains (Chellapandian et al. 1998).

Attempts have been made to improve production of both glucan and glucansucrase by various species of Leuconostoc mesenteroides and for this different media compositions and fermentation conditions have been used (Kobayashi et al. 1986; Lawford et al. 1979; Lopez & Monsan, 1980).

Strains of Leuconostoc mesenteroides require high sucrose concentration, temperature range from 25-30°C and an initial fermentation pH of 7.0 for maximum enzyme production (Tsuchiya et al. 1952; Santos et al. 2000). Moreover, the rate of the cell death follows an exponential decay-law for different values of pH. The optimum pH for cell survival has been found to be pH 5.0, whereas cell death occurs rapidly at extreme pH values (Kim et al. 2000). The presence of manganese, magnesium and calcium salts in the media affects the enzyme activity and increases the glucan yield (Qader et al. 2001). Sugar beet molasses and wheat bran have been used as a carbon source for enzyme production (Behravan et al. 2003).

The objectives of this study were to discover and identify the most potential glucansucrase producing bacterium from locally isolated strains. Glucansucrase and glucan from Leuconostoc mesenteroides have gained worldwide research interest for their diverse properties and potential industrial uses. In this article we have reviewed the factors
contributing to the optimization of conditions for optimal glucansucrase production using a locally isolated strain of L. mesenteroides A1.

**MATERIALS AND METHODS**

Isolation and screening of strain: Bacterial culture was isolated from Brassica oleracea var Capitata L. (Cabbage) purchased from local market using enrichment media technique. Initially the culture was cultivated on MRS medium (BioM Laboratories, USA). The isolates were screened for dextran producing strains by inoculating in medium containing: (g l⁻¹): Sucrose, 50.0; Tryptone, 10.0; Yeast extract, 1.0; K₂HPO₄, 2.5; the pH was adjusted at 7.0 and autoclaved at 121°C for 15 minutes. After autoclaving 0.005 % sodium azide was added aseptically. Bacterial strain was selected showing highly viscous slimy growth on sucrose agar plate.

Strain identification: Leuconostoc mesenteroides A1 was selected for optimization and characterization due to high enzyme activity and dextran producing characteristic. The bacterial strain A1 was isolated and identified by the usual methods (Holt, 1994) and was maintained on sucrose broth medium at 4°C.

Culture media and growth conditions: For fermentation purpose, the organism was grown at 25°C in a medium containing (g l⁻¹): Sucrose, 25.0; Bacto-peptone, 5.0; yeast extract, 5.0; K₂HPO₄, 15.0; MnSO₄. H₂O, 0.01; NaCl, 0.01; MgSO₄.7H₂O, 0.01; CaCl₂.2H₂O, 0.1. The pH of the medium was adjusted to 7.5 before sterilization at 121°C for 15 minutes.

Enzyme isolation: Sterile sucrose broth (10 ml) was inoculated by a loopful culture of L. mesenteroides A1 and incubated at 25°C for 24 hours. This 10 ml of 24 hours old culture was then transferred into 90 ml of same broth medium and incubated at 25°C for 8 hours. After incubation, the cell free supernatant containing the enzyme was obtained by centrifugation at 35000xg for 15 min at 0°C and was stored at -20°C.

Enzyme assay: Glucansucrase activity was determined by measuring the reducing sugar (Kobayashi & Matsuda, 1974). Units of glucansucrase activity are represented in DSU. “One unit of enzyme activity was defined as the enzyme quantity that converts 1.0 milligram of sucrose into fructose and glucan in 1.0 hour at 35°C using citrate phosphate buffer pH 5.00” (Lopez & Monsan, 1980).

Protein assay: Total protein of the cell-free supernatant was determined by the Lowry’s method using bovine serum albumin as a standard (Lowry et al. 1951).

Effect of time, substrate concentration, temperature, and pH on glucansucrase production:

Different conditions like time course, pH, substrate and temperature was optimized for maximum glucansucrase production in fermentation medium. For this purpose, culture medium was incubated for different time intervals (0-48 hours) and for the determination of glucansucrase productivity; total protein, glucansucrase activity, final pH of fermented culture broth and wet cell mass were determined.

After time course, sucrose concentration for the optimum enzyme production was varied from 0.5% to 3.5% in the culture media. For optimum temperature fermentation broths were incubated at various temperatures ranging from 15°C to 45°C with an increment of 5°C. Where as for pH optima, pH of the culture broths was adjusted from 4.5-9.5 by the increment of 0.5 before autoclaving.

Measurements of all the experiments were determined in three independent experiments at least, each performed with three replications.

**RESULTS AND DISCUSSION**

In this study some of the physicochemical parameters affecting bacterial growth were controlled primarily by the changes of environment of the cultural medium such as incubation temperature, pH of medium and addition of sugar to the medium composition. These factors not only influence the cellular growth but also the enzyme production.

**TAXONOMIC ASSESSMENT OF THE ISOLATED STRAIN**

Eleven bacterial isolates identified as Leuconostoc mesenteroides were isolated from the locally available fermented fruits and vegetables (Table 1). The isolates were screened for glucansucrase productivity. The taxonomic assessment of the isolated strain AA1 confirmed the characteristic properties of Leuconostoc mesenteroides and was selected for further studies. Among various strains isolated, bacterial strain AA1 was selected showing highly viscous slimy growth on 5% sucrose agar plate (Fig. 1) whereas slimy, shiny transparent colonies were observed on 2% sucrose agar plate (Fig. 2). The characteristics of the isolated strain are summarized in table 2. A strain of
Leuconostoc mesenteroides AA1 was confirmed according to Bergey’s Manual of Systematic Bacteriology (Holt, 1994).

**Figure 1**
Table 1: strains isolated from various sources and their enzyme activities

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Source</th>
<th>Strains</th>
<th>Enzyme Activity (DSU/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cabbage</td>
<td>AA1</td>
<td>53.5</td>
</tr>
<tr>
<td>2</td>
<td>Cabbage</td>
<td>AA2</td>
<td>23.2</td>
</tr>
<tr>
<td>3</td>
<td>Cabbage</td>
<td>AA3</td>
<td>39.4</td>
</tr>
<tr>
<td>4</td>
<td>Cauliflower</td>
<td>AA4</td>
<td>22.5</td>
</tr>
<tr>
<td>5</td>
<td>Cauliflower</td>
<td>AA5</td>
<td>11.3</td>
</tr>
<tr>
<td>6</td>
<td>Cauliflower</td>
<td>AA6</td>
<td>21.3</td>
</tr>
<tr>
<td>7</td>
<td>Cauliflower</td>
<td>AA7</td>
<td>10.6</td>
</tr>
<tr>
<td>8</td>
<td>Grape</td>
<td>AA8</td>
<td>42.5</td>
</tr>
<tr>
<td>9</td>
<td>Grape</td>
<td>AA9</td>
<td>26.5</td>
</tr>
<tr>
<td>10</td>
<td>Apricot</td>
<td>AA10</td>
<td>11.6</td>
</tr>
<tr>
<td>11</td>
<td>Apricot</td>
<td>AA11</td>
<td>35.0</td>
</tr>
</tbody>
</table>

**Figure 2**
Table 2: Morphological and Physiological Characteristics of the Isolated Strain of AA1

**SELECTION OF MEDIUM**
After isolation of L. mesenteroides AA1, the medium was selected for maximum glucansucrase productivity. Table-3 shows the compositions of four different media used. The experimental culture medium-4 was compared with previously reported medium-1 (Lopez & Monsan, 1980), medium-2 (Tsuchiya, 1952) and medium-3 (Qader et al. 2001). Among four different media prepared, medium-4...
supported maximum extracellular glucansucrase production. When this experimental medium-4 was compared with the other reported media it was observed that this medium supported maximal enzyme production. This medium is not previously reported and was designed to achieve a greater induction of glucansucrase from \textit{L. mesenteroides AA1} (Table 4). In this medium 53.0 DSU/ml/hr activity with a specific activity of 20.3 DSU/mg was recorded which is 5.4, 4.6, and 1.6 times higher than medium-1, medium-2 and medium-3, respectively. This medium was supplemented with varying concentration of mineral ions along with the basic nutrient constituents, which had a positive effect on enzyme productivity. Previously the basic constituents, concentration of sucrose, yeast extract, and \textit{K}_2\textit{HPO}_4 were chosen as critical variables to improve enzyme production by different strains of \textit{L. mesenteroides} (Chellapandian et al. 1998; Kitaoka & Robyt, 1998; Rodrigues et al. 2003).

**Figure 5**
Table 3: Media Compositions for Maximum Glucansucrase Production by AA1

**Table 3: Media Compositions for Maximum Glucansucrase Production by AA1**

<table>
<thead>
<tr>
<th>Media composition (g %)</th>
<th>Medium 1</th>
<th>Medium 2</th>
<th>Medium 3</th>
<th>Medium 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Bacto Peptone</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>\textit{K}_2\textit{HPO}_4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>\textit{NaCl}</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>\textit{MgSO}_4 \cdot \textit{H}_2\textit{O}</td>
<td>-</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\textit{MgCl}_2 \cdot \textit{H}_2\textit{O}</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>\textit{FeSO}_4 \cdot \textit{H}_2\textit{O}</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>\textit{CaCl}_2</td>
<td>-</td>
<td>-</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td>\textit{KHPO}_4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
</tr>
<tr>
<td>\textit{MgSO}_4 \cdot \textit{H}_2\textit{O}</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

*pH of all the media was adjusted to 7.5 before sterilization*

**Figure 6**
Table 4: Selection of Medium for Maximum Glucansucrase Production

<table>
<thead>
<tr>
<th>Medium</th>
<th>Enzyme Activity (DSU/ml/hr)</th>
<th>Total Protein (mg/ml)</th>
<th>Specific Activity (DSU/mg)</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.80</td>
<td>3.37</td>
<td>2.74</td>
<td>4.34</td>
</tr>
<tr>
<td>2</td>
<td>11.48</td>
<td>3.20</td>
<td>3.58</td>
<td>6.06</td>
</tr>
<tr>
<td>3</td>
<td>34.10</td>
<td>3.11</td>
<td>10.96</td>
<td>5.10</td>
</tr>
<tr>
<td>4</td>
<td>53.00</td>
<td>2.61</td>
<td>20.30</td>
<td>5.15</td>
</tr>
</tbody>
</table>

**OPTIMIZATION OF GLUCANSUCRASE PRODUCTION**

Physicochemical parameters that influence the cellular growth and enzyme production were studied including time course for enzyme production, medium composition, substrate concentration, temperature and pH.

**EFFECT OF TIME COURSE ON BACTERIAL GROWTH AND ENZYME PRODUCTION**

In the first step the time course for maximum enzyme production was studied. Growth time of the bacteria in fermentation broth always plays an important role and it was observed that the cell growth of \textit{L. mesenteroides AA1} and enzyme production is a function of time. The relationship between cell growth and enzyme production during fermentation was observed for \textit{L. mesenteroides AA1} and it was recorded that the biomass increased within 2 hours and reached a maximum at 8 hours of incubation with a wet cell mass of 0.85g/dl. As the wet cell mass increased with time (Fig. 3) maximum enzyme production was observed at the last stage of the exponential phase (53 DSU/ml/hr) and the early stationary phase of the cell growth (8 hour). The stationary phase remained up to 18 hour and was followed by a decline phase. Maximum enzyme production at 8 hours of fermentation is a unique property of this strain. Earlier it has been reported that \textit{L. mesenteroides PCSIR-3} shows maximum glucansucrase production in 18 hours while \textit{L. mesenteroides NRRL B-512F} in 12 hours (Qader et al. 2001).

**Figure 7**
Figure 3: Time course of glucansucrase production and cell growth in fermentation medium

**EFFECT OF SUBSTRATE CONCENTRATION**

Among many factors that influence the formation of bacterial enzyme, presence of particular substrate is known to be the most important factor. \textit{L. mesenteroides AA1} was grown in the medium containing sucrose as a carbon source to determine its effect on induction of extracellular glucansucrase. When sucrose
Production of Commercially Important Glucansucrase from a Newly Isolated Strain of Leuconostoc mesenteroides AA1

color concentration was varied from 0.5% to 3.5% during cultivation maximum yield was achieved at 2.5% (Fig. 4). 2.5% of substrate concentration greatly influenced maximum glucansucrase production. During cultivation, the bacterial cells multiply and use sucrose as a carbon source. It has been reported that approximately 85% of sucrose is consumed during glucansucrase production and feeding of sucrose to the culture, increases the yield of glucansucrase up to certain limit. Further increase of sucrose concentration in medium is not utilized by the organism as a carbon source but is consequently accumulated in the medium (Lopez & Monsan, 1980; Neely & Nott, 1962). It has also been reported that the production of glucansucrase gradually declines due to the formation of glucan in the medium that could increase the viscosity of the culture medium and this viscous material retards cells duplication and enzyme production (Lopretti et al. 1999; Michelena et al. 2003). Same was noticed for L. mesenteroides AA1 and it was observed that above this concentration the cells were not easily separated due to the increase in the viscosity of the cultivation medium. Sucrose concentration was varied for production of glucansucrase from L. mesenteroides NRRL B512 (f) and showed that 4% sucrose in culture medium was ideal (Santos et al. 2000). It has also been reported that 2% sucrose is required for maximum glucansucrase production in the cultivation media (Qader et al. 2001; Lopretti et al. 1999; Michelena et al. 2003).

Figure 4
Figure 4: Effect of substrate concentration on glucansucrase production

**EFFECT OF TEMPERATURE**
Temperature may affect the metabolic pattern, nutritional requirements, and composition of bacterial cells. In addition, temperature also affects the rates of all cellular reactions. When L. mesenteroides AA1 was cultivated at various temperatures maximum enzyme production was observed at 25°C and the results showed that the strain is temperature sensitive and enzyme is thermo labile at higher temperatures (Fig. 5). After 25°C a mark decline in glucansucrase production was observed i.e. at 30°C, 55% less enzyme production was observed and at 45°C only 4 DSU/ml/hr activity was examined. It was also observed that L. mesenteroides AA1 is not capable of producing glucansucrase at temperature below 15°C.

Different temperature maxima for glucansucrase production have been reported previously (Santos et al. 2000; Itaya & Yamatoto, 1975; Lopez-Mungia et al. 1998; Monsan et al. 1997).

**Figure 5**
Figure 5: Effect of temperature on glucansucrase production

**EFFECT OF PH**
Enzyme production was also analyzed at different pH values and the optimum glucansucrase production by L. mesenteroides AA1 was achieved when the pH of the cultivation medium was adjusted to 7.5 (Fig. 6). Glucansucrase production was found maximum at pH 7.5 (53.0 DSU/ml/hr) while 31.14 and 20 DSU/ml/hr were detected at pH 7.0 and 8.0, respectively. At slight acidic and alkaline pH values, enzyme production was retarded and L. mesenteroides AA1 failed to produce extracellular glucansucrase at pH 5.0 and 9.0. At these extreme pH conditions morphological and physiological changes occur in the cell that renders enzyme production. Earlier various pH maxima have been reported for the fermentation media for glucansucrase production (Tsuchiya et al. 1952; Lopretti et al. 1999; Lopez-Mungia et al. 1998; Dols et al. 1998). The
Results show that the production of the enzyme depends, inter alia, on pH.

**Figure 10**

Figure 6: Effect of pH on glucansucrase production

**CONCLUSION**

The experimental data of culture conditions optimization for Leuconostoc mesenteroides AA1 is presented in this work. The newly isolated strain Leuconostoc mesenteroides AA1 showed higher extracellular glucansucrase production. In conclusion it seems highly probable that this extracellular glucansucrase is significant from an industrial perspective. It is therefore suggested that Leuconostoc mesenteroides AA1 can be used for the elaboration of the glucansucrase on a large scale, which can allow the commercial production of glucon in a cell free bioreactor.

**References**


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