THE EFFECT OF CALCIUM IONS AND TEMPERATURE ON THE PRODUCTION, ACTIVITY AND STABILITY OF DEXTRANSUCRASE FROM THE NEWLY ISOLATED STRAIN LEUCONOSTOC MESENEROIDES PCSIR-4

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Dextranusscrase [E.C. 2.4.1.5] from Leuconostoc mesenteroides species catalyzes the formation of dextran from sucrose. The newly isolated Leuconostoc mesenteroides PCSIR-4 strain showed maximum enzyme yield in a fermentation medium containing 0.005% CaCl$_2$; the amount of dextranusscrase was 2.5 times higher than in the absence of calcium ions. The maximum enzyme concentration was obtained when the culture was incubated at 25°C, while the activity of the extracellular enzyme reached a maximum at 35°C. The highest thermal stability of the extracellular enzyme was achieved at 30°C. When the enzyme was kept at 50°C the enzyme activity was entirely lost within 240 min. The presence of calcium ions in the culture medium led to an increased enzyme stability and to the preservation of the enzyme activity up to 4 months.

Key words: dextranusscrase, Leuconostoc mesenteroides, dextran, calcium chloride.

INTRODUCTION

Dextranusscrase [E.C. 2.4.1.5] is an industrially important enzyme produced extracellularly by Leuconostoc mesenteroides. The expression of dextranusscrase in L. mesenteroides is inducible (1). This glucosyltransferase catalyzes the formation of dextran from sucrose, with fructose as a by-product (2, 3). Monchois et al. (4, 5) suggested that this is an extracellular enzyme. Recently, it has been observed that many strains of L. mesenteroides produce two types of enzyme, one extracellular and another intracellular. The intracellular enzyme is confined within the cell and can be also used for dextran production.

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Depending upon the *L. mesenteroides* strain, dextransucrase produces either water-soluble or water insoluble dextran (6, 7). Dextran is a high molecular mass glucan widely used in food, pharmaceutical, cosmetic, agricultural and photographic industries. Dextran fractions of specific molecular weights are used for flocculation, stabilization, lyophilization, freeze-drying, and as protective colloids in blood-expanders (8). Crossed linked dextrans are widely used in research and industries for separation purposes.

Different factors play important roles in maximum dextransucrase yielding. Different medium compositions and fermentation conditions have been used by workers to produce dextran and dextransucrase (9, 10). Maximum dextransucrase activity has been obtained by Combes and coworkers for moderate conditions of stirring and aeration (11). Santos and coworkers have found an optimum growth temperature of 25°C to 30°C for genera *Leuconostoc* (12), but Lopretti and coworkers have used a temperature of 35°C for *L. mesenteroides* growth (13). Different workers have also reported that calcium ions and temperature play an important role in the production as well as in the stability of dextransucrase from *L. mesenteroides* NRRL B 512. It has been suggested that the presence of manganese, magnesium and calcium ions in the medium not only affects the enzyme activity, but also increases the dextran yield (10).

We describe in this paper the optimization of growth conditions to obtain a maximum production and stability of dextransucrase in cell-free filtrates.

**MATERIAL AND METHODS**

**STRAIN ISOLATION AND PREPARATION OF INOCULUM**

The *L. mesenteroides* strains were isolated using sucrose broth medium (10) from different fermented vegetables, such as cauliflower, pumpkin, carrot, cabbage and tomato. Using Holt’s method (14) the different strains of *L. mesenteroides* were isolated and identified. All the strains were maintained on sucrose broth medium at 4°C.

For fermentation purpose the culture was grown in a medium containing (g/l): sucrose (20.0), bacto-peptone (5.0), yeast extract (5.0), K₂HPO₄ (15.0), MnSO₄·H₂O (0.01), NaCl (0.01), MgSO₄·7H₂O (0.01) and CaCl₂·2H₂O (0.05), at a pH of 7.5, as we previously described (10).

Ten ml of sterile sucrose broth was inoculated by a loop-full of growing culture of *L. mesenteroides* at 26°C for 24 h. The culture was then transferred into 90 ml of sterile broth medium and incubated again for 24 h at 26°C. This inoculum (100 ml) was used for enzyme production.
PRODUCTION AND STORAGE OF DEXTRANSUCRASE

Sterile sucrose broth medium (900ml) was inoculated with 100 ml inoculum and incubated for 18 h at 26°C. The crude enzyme was then obtained by centrifugation of fermented broth at 15,000 rpm for 15 min at 0°C, and was stored at –20°C.

Calcium ions were incorporated into the medium as calcium chloride salt. Five media (A, B, C, D and E) were prepared, having various concentrations of calcium chloride (from 0.005% to 0.02%).

The extracellular crude enzyme present in cell-free fermentation broth with 2% sucrose was distributed in 1.5 ml polypropylene Treff micro tubes and kept at room temperature (30°C), at 4°C or at –18°C. At different time intervals the enzyme activity was assayed.

ENZYME ASSAY

The enzyme activity was determined in the cell-free supernatant by the method of Kobayashi and Matsuda (15). One unit of dextranucrase (DSU) activity is defined as the enzyme quantity that converts one mg of sucrose into fructose and glucan in one hour under specific conditions (16).

Total protein of the cell-free filtrate was determined by the Lowry method (17) with bovine serum albumin as a standard.

RESULTS

Figure 1 shows that incorporation of calcium ions in the fermentation medium produced more dextranucrase than the media without calcium ions. Table 1 shows that medium-B ensured maximum enzyme production (108.26 DSU/ml/h) as compared to the other media containing calcium chloride.

![Fig. 1. – Time course of dextranucrase production in culture media with and without calcium ions.](image-url)
Figure 2 depicts the effect of temperature on dextran sucrase production and shows that maximum dextran sucrase production was achieved when cultivation medium was incubated at 25°C. A decline in dextran sucrase production was observed for greater values of temperature (30–45°C).

![Figure 2](image_url)

Fig. 2. – Effect of temperature on the production of *L. mesenteroides* PCSIR-4 dextran sucrase.

The maximum enzyme activity in the cell-free filtrate (crude enzyme) was achieved, for *L. mesenteroides* PCSIR-4, at 35°C (Figure 3).

![Figure 3](image_url)

Fig. 3. – Effect of temperature on the activity of extracellular dextran sucrase.

The crude enzyme of *Leuconostoc mesenteroides* PCSIR-4 was stored at different temperatures and enzyme activity was determined at various time
intervals. The highest thermal stability was registered when the enzyme was kept at 30°C, as compared to 40 and 50°C. As the temperature increased, the thermal stability decreased with time (Figure 4). The storage stability data are shown in Figure 5. The enzyme was more stable at −18°C as compared to 4°C and 30°C.

Fig. 4. – Thermal stability of *L. mesenteroides* PCSIR-4 dextransucrase.

Fig. 5. – Storage stability of extracellular dextransucrase at different temperatures.
DISCUSSION

EFFECT OF CALCIUM IONS ON DEXTRANSUCRASE PRODUCTION

Several authors have reported the use of calcium chloride as a source of calcium ions in dextransucrase producing media and have observed that calcium ions play a very important role in enzyme stabilization. Various concentrations of calcium ions have been also used for thermal stabilization of immobilized glucanotransferases (18). A concentration of 0.005% calcium chloride was used for production of \textit{L. mesenteroides} NRRL B-1299 dextransucrase (19), and Lopez-Munguia and coworkers also used 0.005% calcium chloride in the fermentation media (1). On the other hand, inclusion of 0.002% calcium chloride in culture media was recommended by Dols and colleagues for maximum production and stability of dextransucrase from \textit{L. mesenteroides} NRRL B-1299 (20). Kim and Robyt suggested the use of 0.0015% calcium chloride for enzyme production from \textit{L. mesenteroides} B742 (21). In our experiments on \textit{L. mesenteroides} PCSIR-4, incorporation of calcium ions in the fermentation medium produced maximum dextransucrase, as compared to the medium without calcium ions. Enzyme production increased up to 2.5 times when medium was supplemented with calcium ions (Figure 1), the maximum enzyme production being obtained for 0.005% calcium chloride (Table 1). Previously, we have reported that incorporation of 0.005% calcium chloride helps \textit{L. mesenteroides} PCSIR-4 in maximum production of high molecular weight dextran (22).

Table 1

<table>
<thead>
<tr>
<th>Medium</th>
<th>CaCl₂ (%)</th>
<th>Enzyme activity (DSU/ml/h)</th>
<th>Final pH</th>
<th>Total protein (mg/dl)</th>
<th>Wet cell mass (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>53.34</td>
<td>5.37</td>
<td>259.00</td>
<td>0.504</td>
</tr>
<tr>
<td>B</td>
<td>0.005</td>
<td>108.26</td>
<td>5.21</td>
<td>301.20</td>
<td>0.527</td>
</tr>
<tr>
<td>C</td>
<td>0.010</td>
<td>83.25</td>
<td>5.24</td>
<td>328.30</td>
<td>0.529</td>
</tr>
<tr>
<td>D</td>
<td>0.015</td>
<td>74.15</td>
<td>5.23</td>
<td>322.10</td>
<td>0.621</td>
</tr>
<tr>
<td>E</td>
<td>0.020</td>
<td>69.32</td>
<td>5.26</td>
<td>325.30</td>
<td>0.635</td>
</tr>
</tbody>
</table>

EFFECT OF TEMPERATURE ON DEXTRANSUCRASE PRODUCTION

The \textit{L. mesenteroides} strains are very often grown at temperatures below the optimal growth temperature to prevent dextransucrase inactivation (23). While some authors reported the highest dextransucrase production in the fermentation media occurring at a temperature of 27°C (24), others found maximum dextransucrase production being achieved at a temperature of 30°C (1, 25). Cortezi
and coworkers reported a maximum dextranase production in fermentation media at temperatures between 29°C and 31°C for *L. mesenteroides* FT045B and suggested that these temperature values might be more favourable for enzyme production than the traditional cultivation conditions (26). We obtained a maximum dextranase production in fermentation media at a temperature of 25°C. A reduction in enzyme production was obtained at temperatures above 40°C. The strain *L. mesenteroides* PCSIR-4 was not capable of producing dextranase at temperatures below 15°C during fermentation in culture media (Figure 2).

**EFFECT OF TEMPERATURE ON EXTRACELLULAR DEXTRANASE ACTIVITY**

Kim and Robyt have found that mutants of *L. mesenteroides* B-742 constitutive of dextranase have an optimum temperature of 35°C (21). They also reported that the optimum temperature for dextranase from the strain *L. mesenteroides* B-742CB, prepared on both glucose and sucrose, showed a higher temperature maximum (45°C). Other workers reported two forms of dextranase for *L. mesenteroides* B-1355, one having an optimum enzyme activity at 45°C and the other between 35°C and 40°C (27). We obtained a maximum enzyme activity of the cell-free filtrate (crude enzyme) of *L. mesenteroides* PCSIR-4 at a temperature of 35°C (Figure 3). When the temperature increased from 35°C to 40°C, the enzyme activity suddenly dropped four times and no enzyme activity was observed at 50°C.

**THERMAL STABILITY OF EXTRACELLULAR DEXTRANASE**

It has been reported that about 98% of the activity of *L. mesenteroides* IBT-PQ dextranase is lost within 125 min at 40°C, while the activity loss at 30°C is about 2–4% after 125 min (1). It has been also reported that the thermal stability of dextranase depends upon the carbon sources in the fermentation medium. Thus, dextranase from *L. mesenteroides* B-1299CB mutant shows the highest temperature stability when sucrose is the carbon source (28). We observed for dextranase from *L. mesenteroides* PCSIR-4 that stability varied with temperature, the variation being time-dependent. The enzyme lost 26, 86 and 100% of its activity when was stored at 30°C, 40°C and 50°C respectively, for 240 min (Figure 4).

**STORAGE STABILITY OF EXTRACELLULAR DEXTRANASE**

Ajongwen *et al.* reported that about 50% of the activity of the enzyme from *L. mesenteroides* NRRL B 512F was lost within 13 days when stored at 5°C and that the rate of activity was reduced when the enzyme solution was stored between
–12°C and –15°C (29). Lopez-Munguia and collab. reported a half-life of two days at 40°C for the soluble enzyme from L. mesenteroides NRRL B-1355 (27) and claimed that this stability, expressed as glucosyltransferase activity, was higher than that reported by Miller and Robyt (30) and Kaboli and Reilly (31). The activity and stability of L. mesenteroides NRRL B 512F have been also studied for various concentrations of organic solvents and, concerning the stability of the enzyme, it appears that the enzyme is more stable at 4°C than at 30°C (32). Our data presented in Figure 5 clearly indicate a sharp decline (about 27% loss) of the activity of dextransucrase from L. mesenteroides PCSIR-4 within two days of storage at room temperature (30°C). The activity loss was of 22% at 4°C, while at −18°C the activity loss was low (6%). The enzyme activity further declined for all temperatures, but very quickly at room temperature. Only 30% of the activity was preserved after two weeks, and the complete loss of activity was noted after 28 days of storage at room temperature. Residual activities of 30% and 40% were found at 4°C and –18°C respectively, even after four months. Our results show that dextransucrase from L. mesenteroides PCSIR-4 is more stable at 30°C than that of any other strain, having a half-life of 3 days at this temperature. We can conclude that dextransucrase from this strain is more stable when stored at –18°C compared to storage at 4°C or 30°C.

The results of this study offer evidence that calcium ions not only increase dextransucrase production but also provide stability to this enzyme. Our results further demonstrate that maximum production of this enzyme is attained when fermentation is carried at optimum temperature.

REFERENCES

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9. Dextranucrase from Leuconostoc mesenteroides PCSIR-4


