# Influence of Metal ions, Surfactants and Organic Solvents on the Catalytic Performance of Levansucrase from *Zymomonas mobilis* KIBGE-IB14

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**Abstract:** A significant progress has been made in discovering and developing new bacterial polysaccharides producing enzymes possessing extremely functional properties. Levan is a natural polymer of fructose linked by β (2→6) glycosidic bond which is produced by transfructosylation reaction in the presence of levansucrase. Among wide range of microorganisms, *Zymomonas mobilis* is considered as the most promising candidate for the production of extracellular levansucrase. It has potential applications in multiple industries from pharmaceutics, cosmetics to food industries. Determination of levansucrase characteristics is necessary to increase its industrial applications. This concept has directed much interest towards enzyme characterization by observing its effects against different chemicals. The present investigation focused on the characterization of levansucrase by observing its behavior with reference to different metal ions, surfactants and organic solvents. The results showed that these chemicals acted as activators, inhibitors or stabilizers. In metal ions, different activators ($K^+$, $Na^+$, $Cs^+$, $Ba^{+2}$, $Ca^{+2}$, $Cu^{+2}$, $Mg^{+2}$ and $Mn^{+2}$ ) and inhibitors ($Co^{+2}$, $Hg^{+2}$ , $Fe^{+3}$and $Al^{+3}$) were investigated. Among them, $Hg^{+2}$ found to be strong inhibitor as it inhibits enzyme activity by 92% at 1 mM. Non-ionic surfactants i.e. triton X-100, tween-20 and tween-80 considered as stabilizers while anionic surfactant such as sodium dodecyl sulphate (SDS) inhibited the enzyme activity by 11%. Moreover, ethanol and methanol stabilized the enzyme activity while other solvents observed as inhibitors or stimulators.

**Keywords:** Transfructosylation, levan, characterization, sodium dodecyl sulphate (SDS), activators and inhibitors.

## 1. INTRODUCTION

Levansucrase [E.C.2.4.1.10] is a β-D-fructosyltransferase enzyme which is involved in the synthesis of fructan polymer known as levan [1]. Levan is a natural biopolymer of fructose which is derived from sucrose. It consists of linear or branched chains of fructose units which are attached to the sucrose by β (2→6) glycosidic bond in the main linear chain, while branching results from β (2→1) bonds [2, 3]. Levansucrase catalyzes two reactions (i) hydrolysis of sucrose (ii) transfructosylation to form fructose polymers [4]. Levansucrase is classified in glycoside hydrolase (GH) family 68 due to its high hydrolase function [5, 6]. It is one of the industrially promising enzyme that offers a variety of industrial applications as viscosifier, stabilizer, emulsifier, gelling or water binding agent in the field of cosmetics, foods and pharmaceuticals [7]. Apart from this, it can also be used as surface finishing agent, encapsulating agent, sweetener and a carrier of flavor and fragrances [8, 9]. A wide range of microorganisms can produce levansucrase which include *Bacillus* sp., *Streptococcus* sp., *Zymomonas* sp. and *Aspergillus* sp. [10]. Although, levan has potential applications but the amount of levan produced is not equal to the other biopolymers which is mainly due to the inefficiency of producer organism. However, for large scale production of levan, *Z. mobilis* is considered as a potential candidate among many levan producing organisms [11].

It has been reported that the levansucrase from various sources differ widely from each other on the basis of its physico-chemical properties such as molecular weight, stimulator or inhibitor specificity and relative activity by various reactions and optimal conditions for high yield of enzyme [12]. Besides these distinctions, levansucrases obtained from Gram's negative origin display similarities in their characteristic features which mainly include molecular weight, constitutive production as well as amino acid sequence [13-16].

The success of multifarious industrial applications of any enzyme depends on the optimization of catalytic parameters. Therefore, the effect of different activators, inhibitors and stabilizers are very significant for better understanding of enzymatic phenomenon. The current study is designed to investigate the effect of different metal ions (monovalent, divalent and trivalent), organic solvents and detergents (nonionic and anionic) on the activity of levansucrase in order to characterize it for future utilizations.

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## 2. MATERIAL AND METHODS

### 2.1. Bacterial Strain and Culture Conditions

*Z. mobilis* KIBGE-IB14 has been deposited to the NCBI GenBank having accession # HM102366. The strain was maintained in optimized medium containing (%) 15 % sucrose; 0.2 % peptone; 1.0 % yeast extract; 1.5 % $K_2HPO_4$ and 0.01 % $CaCl_2.2H_2O$. The culture was incubated at 35 °C for 24 hours and pH was adjusted up to 6.5.

### 2.2. Levansucrase Assay

The activity of levansucrase was assayed by estimation of glucose liberated from sucrose in transfructosylation reaction according to the glucose oxidase (GOD-PAP) method using glucose as standard [17, 18]. The assay mixture containing 1.0 ml sucrose (0.25 M) in 0.5 ml sodium phosphate buffer (0.1 M, pH 6.0) and 0.5 ml of cell free filtrate (CFF) was incubated at 35 °C for 5.0 minutes. After incubation, the reaction was stopped by adding 0.5 ml NaOH (1N) in the reaction tube. Glucose liberated during the reaction was estimated by standard method at 546 nm. One unit (U) of levansucrase is expressed as the amount of the enzyme that releases 1.0 µM of reducing sugar as glucose per minute under standard assay conditions. Specific activity of the enzyme was expressed as units per mg of protein (U $mg^{-1}$).

### 2.3. Protein Estimation

Protein concentration was measured by Lowry's method [19] using bovine serum albumin (BSA) as standard.

### 2.4. Effect of Metal ions on Levansucrase Activity

Various monovalent ($K^+$, $Na^+$, $Cs^+$), divalent ($Ba^{2+}$, $Co^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Ca^{2+}$, $Ni^{2+}$, $Hg^{2+}$, $Mg^{2+}$) and trivalent ($Al^{3+}$, $Fe^{3+}$) ions were used to investigate their impact on the activity of levansucrase. For this purpose, enzyme was pre-incubated with 1.0, 5.0 and 10.0 mM solutions at 37 °C. After 60.0 minutes of enzyme incubation, these samples were retrieved to conduct their enzymatic activity at 35 °C. All of the observed metal ions required in this process were in the form of chloride salts. The percent relative activity of levansucrase in the presence of different metal ions was calculated by comparing it with control (enzyme untreated with any metal ion). The activity of enzyme was accomplished by standard assay conditions.

### 2.5. Influence of Surfactants on Levansucrase Activity

Levansucrase was incubated by several surfactants (nonionic and anionic) including triton X-100, tween 80, tween 20 and sodium dodecyl sulfate (SDS) with 1.0, 5.0 and 10.0 mM concentrations at 37 °C for 60.0 minutes to evaluate their effect exerted on activity of enzyme. Levansucrase free from any surfactant treatment was taken as control and its value was considered as 100%.

### 2.6. Effect of Organic Solvents on Levansucrase Activity

The influence of organic solvents on levansucrase activity was determined by incubating levansucrase with various organic solvents such as ethanol, methanol, isopropanol, formaldehyde, chloroform, EDTA and DMSO. The different concentrations of solvents were used which include 1.0, 5.0 and 10.0 mM for 60.0 minutes at 37 °C. Enzyme without exposed to any solvent was considered as control and taken as 100%.

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of Monovalent Metal ions on Levansucrase Activity

The chemicals i.e. metal ions are able to create interactions with the active site of enzyme which affects the discipline of activation, inhibition and stabilization in enzyme substrate reactions. The biocatalysts can be represented as activators, inhibitors or stabilizers in the presence of different metal ions having appropriate concentration. The results exhibited that $K^+$, $Na^+$ and $Cs^+$ showed positive effect on enzyme activity at 1 mM concentration as the activity was stimulated by 5%, 2% and 1% respectively (Table **1**). Subsequently, the activity of levansucrase decreased with an increase of metal ions concentration. Gonçalves *et al*. [20] has been found that levansucrase activity from *B. subtilis* Natto CCT 7712 was increased by monovalent cations i.e. $Na^+$, $K^+$ and $Zn^+$ (4 mM to 8 mM).

### 3.2. Influence of Divalent Metal ions on Activity of Levansucrase

Levansucrase reacted completely different in the presence of divalent metal ions as depicted in Table **2**. $Ba^{+2}$, $Ca^{+2}$, $Cu^{+2}$, $Mg^{+2}$ and $Mn^{+2}$ acted as stimulators as they enhanced levansucrase activity by 6%, 25%, 2%, 4% and 2% respectively at 1 mM concentration which

**Table 1: Effect of Monovalent ions on Levansucrase Activity from *Z. mobilis* KIBGE-IB14**

| Metal ions | Relative Activity (%) | | |
|---|---|---|---|
| | 60 minutes | | |
| | 1mM | 5mM | 10mM |
| Control | 100 | | |
| $K^+$ | 105 | 93 | 90 |
| $Na^+$ | 102 | 95 | 92 |
| $Cs^+$ | 101 | 94 | 88 |

was further decreased by increasing the concentration of metal ions. While a little effect was noticed with $Ni^{+2}$ and $Zn^{+2}$. However, different results have been reported by Ammar *et al*., 2002 that $Zn^{+2}$ (5 mM) showed inactivation of levansucrase from *Bacillus* sp. TH4 activity [21]. Two divalent ions such as $Co^{+2}$ and $Hg^{+2}$ showed inhibitory effect on levansucrase activity by 24% and 92% at 1 mM concentration that declined more with the increase of concentration. While $Hg^{+2}$ was considered as the strong inhibitor because it inhibited the enzyme activity by 98% at 10 mM concentration. It has been reported that heavy metals acted as non-competitive inhibitors as they bind with enzyme other than active site because of its conformational changes. This change might be due to the cleavage of disulfide bonds in cysteine residues where they bind with sulfur of thiol (SH) group due to similar electronegativity as that of hydrogen. Thus, the enzyme becomes inactive by the replacement of hydrogen which alters the conformation of its active site [22, 23]. In the case of amyloglucosidase, heavy metals such as $Cu^{+2}$, $Fe^{+2}$, $Hg^{+2}$, $Ni^{+2}$ and $Zn^{+2}$ reduced enzyme activity by 30%, 60%, 40%, 11% and 20% respectively at 10 mM [24]. In contradiction, xylanase from *Bacillus altitudinis* DHN8 has been reported for enzyme activation in the presence of $Mn^{2+}$ and $Ca^{2+}$ metal ions with 126% and 168% relative activity respectively [25]. Moreover, In the case of protease, $Ca^{2+}$ and $Mg^{2+}$ enhanced the enzyme activity by 4.5% and 6.92% respectively while $Zn^{2+}$ and $Cu^{2+}$ restrained the protease activity by 61.88% and 73.42% respectively [26].

### 3.3. Influence of Trivalent Metal ions on Activity of Levansucrase

Two trivalent ions ($Fe^{3+}$ and $Al^{3+}$) were used in current research to notice their impact on catalytic performance of levansucrase. Results exhibited that $Fe^{+3}$and $Al^{+3}$ showed negative effect on levansucrase activity as they inhibited the activity of enzyme by 12% and 18%. It has been reported that the activity of levansucrase was inhibited by $Al^{+3}$ (88%) while $Fe^{+3}$ did not show any considerable effect [27].

### 3.4. Effect of Nonionic Surfactants on Levansucrase Activity

The surfactants are acknowledged as an important environmental factor that induce their effect on the activity of enzyme. This study represents an attempt to

**Table 2: Effect of Divalent ions on Activity of Levansucrase Obtained from *Z. mobilis* KIBGE-IB14**

| Metal ions | Relative Activity (%) | | |
|---|---|---|---|
| | 60 minutes | | |
| | 1mM | 5mM | 10mM |
| Control | 100 | | |
| $Ba^{+2}$ | 106 | 99 | 87 |
| $Ca^{+2}$ | 125 | 133 | 136 |
| $Co^{+2}$ | 76 | 68 | 54 |
| $Cu^{+2}$ | 102 | 89 | 76 |
| $Hg^{+2}$ | 8 | 4 | 2 |
| $Mg^{+2}$ | 104 | 96 | 88 |
| $Mn^{+2}$ | 102 | 98 | 90 |
| $Ni^{+2}$ | 98 | 94 | 89 |
| $Zn^{+2}$ | 97 | 92 | 90 |

**Table 3: Trivalent ions Influence on Levansucrase Activity from *Z. mobilis* KIBGE-IB14**

| Metal ions | Relative Activity (%) | | |
|---|---|---|---|
| | 60 minutes | | |
| | 1mM | 5mM | 10mM |
| Control | 100 | | |
| $Fe^{+3}$ | 88 | 80 | 73 |
| $Al^{+3}$ | 82 | 71 | 65 |

find out the effect of nonionic surfactants on the activity of levansucrase from *Z. mobilis* KIBGE-IB14 (Table **4**). According to current investigation, non-ionic detergents i.e. triton X-100, tween-20 and tween-80 did not expressively produce any changes in the activity of levansucrase at all concentrations. This might be due to the fact that these nonionic surfactants did not cause inhibition or inactivation of enzymes as they prevent the aggregation of protein in solution and provide stabilization to protein activity. In line with this observation, one of research group was found similar results in pepsin where triton X-100 and tween-80 acted as stabilizers [27].

### 3.5. Effect of Anionic Detergent on Levansucrase Activity

Different effect has been noted by the influence of anionic detergent on the activity of levansucrase (Table **4**). Anionic detergent i.e. SDS showed slight inhibition on levansucrase activity as it inhibited the enzyme by 11% at 1 mM concentration which gradually decreased as the concentration of surfactant was increased. Hettwer *et al*. [27] revealed that SDS slightly inhibited the levansucrase activity by 73%. It has been reported that this inhibition was due to the ability of all proteins to absorb anionic detergent that denatures its structure by disrupting bonds which ultimately obstructs protein function [28]. Zikmanis *et al*. [29] described the effect of different surfactants of various concentrations on levansucrase activity from *Z. mobilis*.

### 3.6. Influence of Organic Solvents on Levansucrase Activity

The performance of enzyme is affected by organic solvents in a way that it causes alteration in active site of enzyme [30]. It has been reported that solvents can also affect the enzyme activity by disturbing its interaction with substrate and product. This disturbance is due to the alteration of substrate and product concentration in the aqueous layer surrounding the enzyme that hinders the diffusion of product and permeation of substrate [31, 32]. The stability and catalytic activity of levansucrase in water-miscible environment was investigated by incubating the enzyme with different organic solvents in various concentrations (Table **5**). The study exhibited that all solvents did not show any significant effect on levansucrase activity at low concentrations (1 mM) except isopropanol and DMSO that acted as an activator and inhibitor of enzyme respectively. At 1 mM concentration of solvents, isopropanol stimulateed the enzyme activity by 10% while DMSO showed 14% inhibition and this relative percent declined more as the concentration of organic solvents increased. At higher concentrations of solvents (5 mM and 10 mM), the enzyme activity remained almost stable in the case of ethanol and methanol while formaldehyde and chloroform found to be the inhibitors of enzyme. The inhibition of enzyme activity by organic solvents might be due to the penetration of solvents in the active site of enzyme where it causes electrostatic repulsion

**Table 4: Effect of Surfactants (Nonionic and Anionic) on the Activity of Levansucrase from *Z. mobilis* KIBGE-IB14**

| Surfactants | Relative Activity (%) | | |
|---|---|---|---|
| | 60 minutes | | |
| | 1mM | 5mM | 10mM |
| Control | 100 | | |
| Tween-20 | 100 | 100 | 92 |
| Triton X-100 | 98 | 93 | 88 |
| Tween-80 | 100 | 98 | 90 |
| SDS | 89 | 80 | 75 |

**Table 5: Impact of Organic Solvents on the Activity of Levansucrase from *Z. mobilis* KIBGE-IB14**

| Solvents | Relative Activity (%) | | |
|---|---|---|---|
| | 60 minutes | | |
| | 1mM | 5mM | 10mM |
| Control | 100 | | |
| DMSO | 86 | 72 | 50 |
| Ethanol | 100 | 100 | 96 |
| Methanol | 100 | 98 | 94 |
| Isopropanol | 110 | 96 | 87 |
| Formaldehyde | 96 | 82 | 76 |
| Chloroform | 100 | 95 | 84 |

between enzyme and substrate that renders its interaction [30]. Previously reported the different effects of various organic solvents (1,4-dioxane, acetone, acetonitrile and DMSO) on activity of levansucrase from *B. subtilis* [33].

## 4. CONCLUSION

The present study reflects the effect of several metal ions, surfactants as well as organic solvents on the catalytic activity of levansucrase obtained from *Z. mobilis* KIBGE-IB14. Every chemical influences the enzyme's activity by its own specific manner instead of similar conditions and concentrations used. The observations of this research revealed that inhibition or activation of enzyme activity do not only depend upon nature of chemical but on the possible synergism between chemical and enzyme.

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