JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

OPTIMIZATION OF PROCESS PARAMETERS FOR MAXIMUM PRODUCTION AND CHARACTERIZATION OF DEXTRANSUCRASE FROM NEWLY ISOLATED *ACETOBACTER TROPICALIS*

Nisha[1]*, *Wamik Azmi*[1]

Address(es): Nisha, PhD
Department of Biotechnology, Himachal Pradesh University, Shimla, 171005, India.

*Corresponding author: sem.nisha24@gmail.com

doi: 10.15414/jmbfs.2018.7.6.628-635

ARTICLE INFO

Received 4. 4. 2018
Revised 2. 5. 2018
Accepted 16. 5. 2018
Published 1. 6. 2018

Regular article

OPEN ACCESS

ABSTRACT

The potent producer *A. tropicalis* was isolated and selected on the basis of high dextransucrase activity. Different physicochemical parameters for *A. tropicalis* dextransucrase production have been optimized. *A. tropicalis* showed maximum dextransucrase activity (4.58 U/mL) when incubated for 8 h at 25 ºC in medium having initial pH 7. The 2^6 technological combinations were also designed to study the effect of physical and chemical parameters in combination which proved to be very satisfactory on economical point of view. The selected combination gave 21.2 folds increase in dextransucrase activity over the basal medium (0.23 U/mL) and more activity than the medium formed after studying the effect of individual physicochemical parameters. Optimum reaction conditions for maximum dextransucrase activity were found to be 25 mM sodium acetate buffer with pH 5.5, reaction temperature 37 ºC and sucrose concentration 2% (w/v). Optimization of reaction conditions resulted in maximum 12.2 U/mL dextransucrase activity. This activity was higher than the activity reported previously for other strains. Crude dextransucrase *A. tropicalis* showed K_m=29.86 mM and V_{max}=7.0 U/mL . This is the first report on dextransucrase production by *A. tropicalis.*

Keywords: *Acetobacter tropicalis,* dextransucrase, dextran, technological combinations

INTRODUCTION

Dextransucrase is an extracellular enzyme produced by various species of *Streptococcus* **(Bailey and Oxford, 1958; Ul Qader *et al.*, 2005)**, *Leuconostoc* **(Naessens *et al.*, 2005; Yamaner *et al.*, 2010; Hashem *et al.*, 2012; Miljkovic *et al.*, 2017)** and *Lactobacillus* **(Yamaner *et al.*, 2010; Rania *et al.*, 2013).** Other species reported for dextransucrase production are *Weissella confusa*, *Pediococcus pentosaceus*, *Weissella cibaria* JAG8 and *Oenococcus kitaharae* DSM 17330 **(Yamaner *et al.*, 2010; Patel *et al.*, 2011; Rao and Goyal, 2013; Vuillemin *et al.*, 2018).** Dextranuscrase synthesizes dextran by catalyzing the transfer of D-glucopyranosyl residue from sucrose to the reducing end of the growing dextran which is covalently attached to the active site of the enzyme **(Leathers, 2002)**. This enzyme-substrate reaction releases fructose as by product. **(Barker and Ajongwen, 1991)**. **Hucker and Pederson (1930)** were the first who reported the production of dextransucrase by strains of *Leuconostoc* species. Jeans and his coworkers reported the synthesis of dextransucrase from different strains of bacteria that were primarily *Leuconostoc* strains **(Jeans *et al.*, 1954)**. *L. mesenteroides* NRRL B-512(F) dextransucrase is the first enzyme used commercially to produce dextran **(Robyt and Walseth, 1979; UL Qader *et al.*, 2005)**. *L. citreum* B/110-1-2 **(Vidal *et al*, 2011)** and *L. mesenteroides* LM-0326 **(Zhang *et al.*, 2008)** are the other strains used commercially.

Dextran $(C_6H_{10}O_5)n$ is the collective term used to a group of bacterial polysaccharides composed of chains of D-glucose units connected by α(1-6) linkages in the linear chain and α(1-2), α(1-3) and/or α(1-4) branch linkages **(Dols *et al.*, 1998)**. The dextran has various commercial applications, generally in the pharmaceutical industry. Most commonly dextran is used as a blood volume expander but, new applications are being considered in food and textile industries **(Koepsell and Tsuchiya, 1952)**. In addition to the dextran production, dextransucrase find more novel applications in the production of oligosaccharides. These oligosaccharides are used in food, feed and cosmetic sectors as stabilizers, bulking agents, immune-stimulating agents and prebiotic compounds **(Remaud-Simeon *et al.*, 2000)**.

Considering the importance of dextransucrase in the pharmaceutical industry, the present study was carried out to develop the suitable process for the production of dextransucrase by newly isolated strain. In this work, many strains having dextransucrase activities were isolated from different soil samples from the various regions of Himachal Pradesh**.** Hyper producer of the dextransucrase was screened and selected for the further study.

MATERIALS AND METHODS

Chemicals

All the chemicals used in the present study were of analytical grade procured from HiMedia Laboratories Pvt. Ltd., Mumbai. The media constituents were of bacteriological grade.

Isolation and screening of dextransucrase positive microorganisms from the soil samples

Dextransucrase producing microorganisms were isolated from the soil samples collected from sugarcane juice extraction sites (Shimla) and sugarcane fields (Bilaspur) and bagasse of sugar mill (Ponta Sahib) from Himachal Pradesh. The soil samples were serially diluted (up to 10^{-8} dilutions) in the sterile physiological saline and 50 µL of each dilution was spread on the sterilized modified MRS agar plates containing % (w/v): Peptone 1.0, yeast extract 0.4, meat extract 0.8, sucrose 2.0, CH_3COONa 0.5, K_2HPO_4 0.2, $MgSO_4.7H_2O$ 0.02, $MnSO_4.2H_2O$ 0.005, agar 2.0 (5.5 pH). The plates were incubated at 37ºC. The microorganisms showing slimy colonies on sucrose rich medium were selected and sub-cultured to obtain pure cultures. The isolates were evaluated for dextransucrase activity in the culture broth. The potent producer of dextransucrase was selected and sent to Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, for identification. The isolate was identified as *Acetobacter tropicalis*

Determination of dextransucrase activity

The reaction mixture (2 mL) containing enzyme (10 µL) and sucrose (2%, w/v) in sodium acetate buffer (25 mM, pH 5.5) was incubated at 37ºC for 30 min. Aliquots (1 mL) from the reaction mixture were analyzed for reducing sugar by DNS (dinitro salicylic acid) method **(Miller, 1959)**. Activity of dextransucrase from *A. tropicalis* was expressed in terms of units (U/mL). The dextransucrase unit is

defined as the amount of enzyme catalyzing the formation of 1 µmol of D-fructose from sucrose in sodium acetate buffer at 37 ºC/mL/min.

Selection of medium and role of inducer

Thirteen different media were tested for the maximum production of dextransucrase by *A. tropicalis.* The initial pH of all media was adjusted to 5.5. The production media were inoculated with *A. tropicalis* seed and incubated at 37ºC for 24 h. Role of inducer (Sucrose 2.0%) in the seed and the production medium for the production of dextransucrase was also studied by preparing combinations of seed and production medium with and without sucrose.

OPTIMIZATION OF PRODUCTION PARAMETERS

The effect of various physical parameters *viz.* initial pH, temperature, rpm (revolution per minute), inoculum size and age of the inoculum was investigated. These parameters were studied in 100 mL of production medium prepared in Erlenmeyer flasks (250 mL). Different chemical parameters (carbon sources, growth supplements, organic nitrogen sources, inorganic nitrogen sources, inorganic salts and metal ions) were also evaluated for maximum dextransucrase production by *A. tropicalis*. In all the cases, growth, final pH of the medium and dextransucrase activity were determined.

DESIGNING 2^6 TECHNOLOGICAL COMBINATIONS ON OPTIMIZED PRODUCTION PARAMETERS

In order to reduce the cost of production medium, technological combinations were designed. For this purpose, instead of one parameter being varied, different combination of optimum and next lower level of optimized parameter were taken into account. The optimized physicochemical factors considered were, medium pH, incubation temperature, carbon source (sucrose), organic nitrogen source (peptone), metal ion ($MnSO_4.2H_2O$) and inorganic salt (CH_3COONa). Total 64 combinations (2^6 factorial) were obtained on the basis of above parameters. The previously optimized medium was considered as control to compare the dextransucrase activity with the different combinations. In each case, growth, final pH and dextransucrase production by *A. tropicalis* were monitored.

CHARACTERIZATION OF CRUDE DEXTRANSUCRASE OF *A. TROPICALIS*

Crude dextransucrase of *A. tropicalis* was characterized for its buffer pH (4-10), Buffer molarity and temperature (20-50 ºC). Effect of sucrose concentration and metal ions on dextransucrase activity was also studied. K_m and V_{max} values form crude dextransucrase of *A. tropicalis* were calculated and thermostability of enzyme at different temperatures was analyzed.

RESULTS AND DISCUSSION

Isolation, screening and selection of dextransucrase hyper-producer

The hyper-producer was identified as *A. tropicalis* with 0.22 U/mL dextransucrase activity. This strain has circular, convex, shiny and non-pigmented colonies. Gram staining of *A. tropicalis* cells confirmed its gram-negative nature with rod shaped structure. On the basis of dextransucrase activity in liquid medium, *A. tropicalis* was selected for the further work.

Selection of medium for maximum production of dextransucrase and role of inducer

Out of 13 media (data not shown here), the medium (pH 5.5) containing (%, w/v) Sucrose 2.0, yeast extract 0.4, peptone 1.0, meat extract 0.8, CH_3COONa 0.5, K_2HPO_4 0.2, $MgSO_4.7H_2O$ 0.02, $MnSO_4.2H_2O$ 0.005 has been found the most suitable for the production of dextransucrase by *A. tropicalis*. The maximum activity obtained with this medium was 0.23 U/mL with 1.7 mg/mL of cell mass. Role of inducer in the seed and the production medium for the production of dextransucrase was also studied. Presence of sucrose in both the seed and production medium was proved to be necessary for the maximum activity of dextransucrase (0.23 U/mL). Rest of the combinations showed negligible/no dextransucrase activity (Table 1).

Table 1 Role of inducer in seed and production medium on the production of dextransucrase by *A. tropicalis*

S.No.	Seed medium	Production medium	Cell mass (mg/mL)	Enzyme activity (U/mL)	Final pH
1	**S+ve**	**S+ve**	**1.7**	**0.23**	**5.2**
2	S+ve	S-ve	1.0	0.01	6.5
3	S-ve	S+ve	1.2	0.06	6.5
4	S-ve	S-ve	0.6	-----	6.9

Legend: S+ve-sucrose added in medium, S-ve- Sucrose not added in medium

OPTIMIZATION OF PRODUCTION CONDITIONS

Optimization of pH

The variation in the pH of production medium greatly affects the uptake of nutrient from the medium this is why, it is important to optimize the pH of the medium. Production medium with wide range of initial pH (4.0-8.0) was inoculated with 24 h old seed culture of *A. tropicalis* (2.0%, v/v) to find out the optimum pH for the maximum production of dextransucrase. The maximum growth (1.72 mg/mL) and dextransucrase production (0.45 U/mL) have been observed at pH 7.0 and on further increase or decrease in the initial pH from the optimum, the enzyme activity was found to decrease gradually (Fig. 1). However, the final pH of the fermentation broth *A. tropicalis* has been found to decrease (5.3). This indicates that acidic conditions were developed due to the production of extracellular enzyme secretion by the cells which ultimately favored the dextran production **(Sarwat *et al.*, 2008)**. Similar results were obtained with strains of *Leuconostoc* where the pH of the growth medium fell down from 6.9 to 4.2 after 32 h of incubation **(Patel and Goyal, 2010)**. Some other studies reported that the dextransucrase production from *L. mesenteroides* spp. shows optimum pH range from 6.7-7.5 **(Leathers, 2002; Sarwat *et al.*, 2008)**. Though, the optimal pH for dextransucrase production has been reported to extend over 5.0-8.0 pH range **(Koepsell and Tsuchiya, 1952; Tong, 1973; Aslop, 1983; Lazic *et al.*, 1993; Santose *et al.*, 2000; Sawale and Lele, 2010, Leathers, 2002; Rania *et al*, 2013)**.

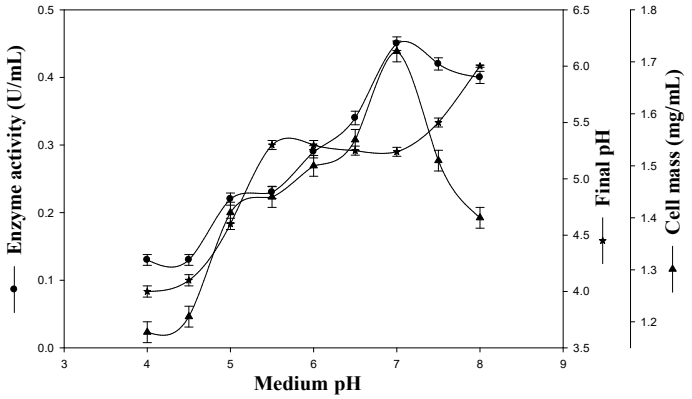

Figure 1 Effect of medium pH on growth and dextransucrase production by *A. tropicalis*

Optimization of incubation temperature

The production medium was inoculated with *A. tropicalis* seed (2%, v/v) and incubated over wide range of temperatures (20 to 55 ºC) for 24 h. The maximum growth (2.6 mg/mL) and dextransucrase (0.58 U/mL) production was observed at incubation temperature of 25 ºC (Fig. 2). However, with further increase in incubation temperature, a decrease in growth as well as enzyme production was observed. Moreover, no growth of the cells of *A. tropicalis* has been observed at incubation temperature at and above 45ºC. *L. mesenteroides* NRRL B512(F) dextransucrase also showed maximum activity (1.9 U/mL) at low incubation temperature, 20 °C **(Santos *et al.*, 2000)**. Whereas, *L. mesenteroides* PCSIR-3 and *L. mesenteroides* CMG713 dextransucrase gave best result at 26 °C and 30 ºC, respectively with enzyme activity of 0.23 U/mL and 0.67 U/mL, respectively **(Ul Qader *et al.*, 2001; Sarwat *et al*, 2008).** In the recent study, maximum activity of *L. mesenteroides* T3 dextransucrase (3.10 U/ml) was obtained at 23 °C **(Miljkovic *et al.*, 2017).**

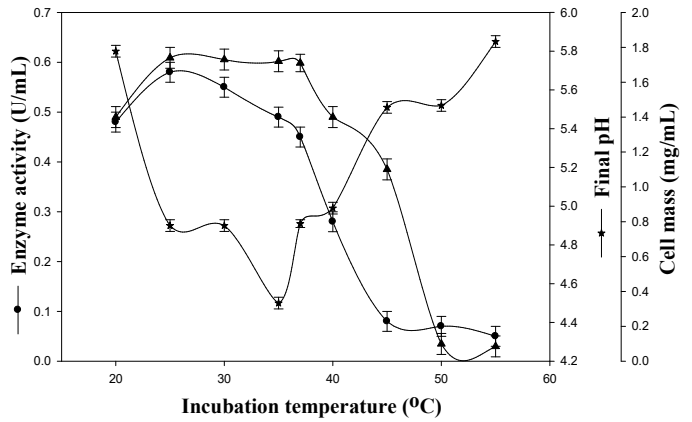

Figure 2 Effect of incubation temperature on growth and dextransucrase production by *A. tropicalis*

Optimization of the inoculum age and of inoculum size

The production media (pH 7.0) were inoculated with the 2.0% (v/v) *A. tropicalis* inoculum of the 6, 9, 12, 15, 18, 21, 24, and 27 h age and incubated at 25 ºC. The maximum enzyme activity (1.35 U/mL) and cell mass (1.75 mg/mL) were obtained with 12 h old inoculum (Fig. 3). Dextransucrase activity in the production medium was found to decrease slightly by use of more and less than 12 h old inoculum. These results gave clear indication that the cells of *A. tropicalis* were metabolically most active at 12 h age. The pH of the fermentation broth was found to decrease from 7.0 to 4.8 which support the dextran accumulation by action of dextransucrase. On the basis of these results, 12 h old (2%, v/v) inoculum was used for the production of dextransucrase from *A. tropicalis*. Cortezi and his co-workers have used 14-16 h old inoculum for dextransucrase production from *L. mesentroides* FT045 B **(Cortezi *et al.*, 2005).**

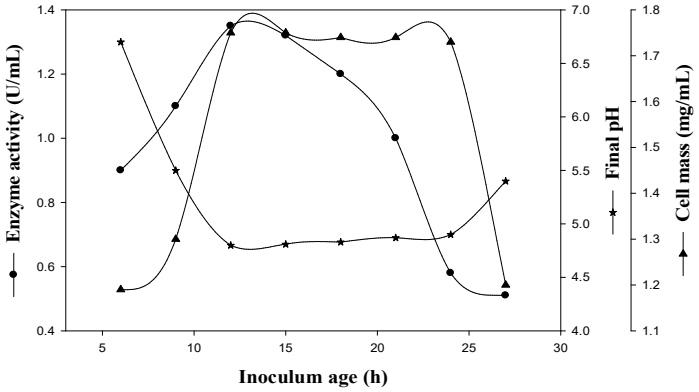

Figure 3 Role of inoculum age on growth and dextransucrase production by *A. tropicalis*

In order to find out the effect of inoculum size on the growth and dextransucrase production by *A. tropicalis*, 0.5-10% (v/v) 12 h old inoculum was added to the production medium. The dextransucrase activity (3.43 U/mL) and growth (1.78 mg/mL) has been found maximum with 1% (v/v) of the inoculum (Fig. 4). The growth reduced with increase in inoculum size up to 3% (v/v) and became almost constant thereafter. This decrease in enzyme production at higher inoculum sizes may be due to competition for nutrients and oxygen in the culture, consequently, failing the enzyme to attain production stage. Hence, for all subsequent experiments, 1% (v/v), 12 h old inoculum was used. Inoculum size and age reported for the production of dextransucrase by *L. mesenteroides* was 10%, 24 h respectively **(UL Qader *et al,* 2005)**. The study by **Sawale and Lele (2010$_a$)** showed 2% (v/v) inoculum size for maximum production of *Leuconostoc* sp. dextransucrase. Whereas, for dextransucrase production by *L. mesenteroides* NRRL B-1149, 6-7 h, 1% inoculum has been reported **(Shukla *et al.*, 2010).**

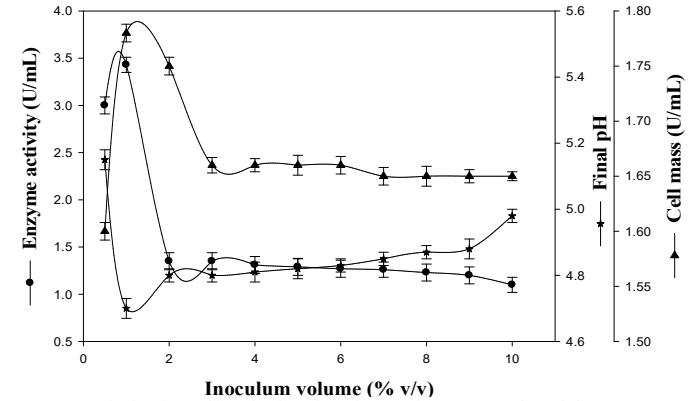

Figure 4 Optimization of incubation time for maximum growth and dextransucrase production by *A. tropicalis*

Optimization of incubation time

Production medium was inoculated with 1% (v/v) seed (12 h old) and incubated at 25 ºC (150 rpm). Samples were withdrawn at regular interval of 2 h and analyzed for final pH, growth and dextransucrase activity. The active cell growth continued up to 8 h (1.79) of incubation and then attained the stationary phase of growth (Fig. 5). pH of the medium had also started decreasing with increasing incubation time due to dextransucrase production. The maximum dextransucrase activity of *A. tropicalis* was found to be 3.83 U/mL at 8 h of cultivation which coincided exactly with that of maximum cell growth. The optimum fermentation time for *L. mesenteroides* FT 045, *L. mesenteroides* CMG713 and *L. mesenteroides* NRRL B-512(F) were reported to be 8 h, 20 h and 6 h respectively **(Cortezi *et al.*, 2005; Sarwat *et al.*, 2008; Honorato and Rodrigues, 2010)**. Whereas, 48 h and 8 h of incubation periods have been reported for maximum dextransucrase production by *Lactobacillus acidophilus* ST76480.01 and *W. confusa* **(Rania *et al.*, 2013; Zafar *et al.*, 2018)**

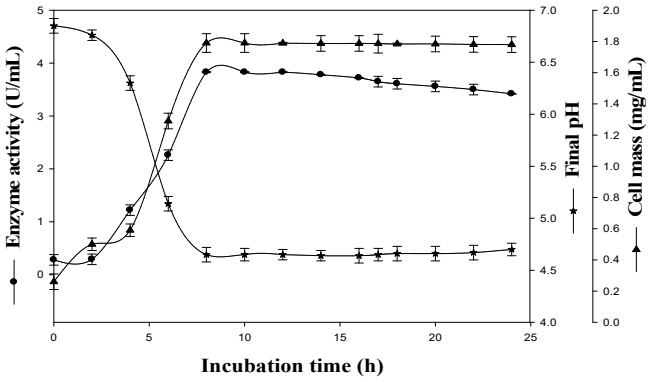

Figure 5 Optimization of incubation time for maximum growth and dextransucrase production by *A. tropicalis*

Optimization of agitation rate

A. tropicalis culture was incubated (25 ºC) at varying rpm in order to find the optimum agitation rate for dextransucrase production. A culture flask set under static condition was considered as control. The maximum dextransucrase activity (3.83 U/mL) and growth of *A. tropicalis* (1.8 mg/mL) was detected at 150 rpm and the further increase in agitation speed lead to decrease in enzyme activity (Fig. 6).

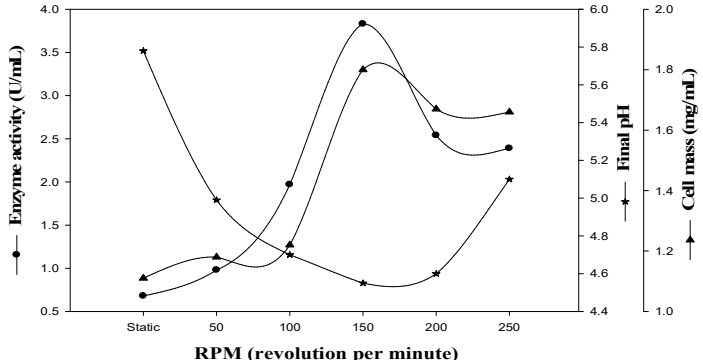

Figure 6 Effect of agitation speed on growth and dextransucrase activity of *A. tropicalis*

These results suggested that 150 rpm agitation speed might generate enough shears for dextransucrase production. Moreover, under static condition, cells grew well but, dextransucrase activity decreased adversely. Some other authors have reported 180 rpm, 150 rpm and 200 rpm **(Dols *et al.*, 1998; Honorato and Rodrigues, 2010; Sawale and Lele, 2010$_a$)** for the production of dextransucrase. Contrary to above given results, *L. mesenteroides* T3 gave best results (3.1 U/mL) at static condition in the recent report (**Miljkovic *et al.*, 2017**).

EFFECT OF INDIVIDUAL CHEMICAL FACTORS ON MAXIMUM GROWTH AND DEXTRANSUCRASE PRODUCTION BY *A. TROPICALIS*

Optimization of substrate

The various carbon sources (mannitol, maltose, sucrose, raffinose, lactose, galactose and starch) with 2% (w/v) concentration were added to production medium (pH 7.0) containing 1.0 % (w/v) peptone. The medium without any carbon source (containing only peptone) was considered as control. Among the different carbon sources tested, maximum enzyme activity (1.19 U/mL) and growth (0.91 mg/mL) was produced with sucrose (Fig. 7). In rest of the carbon sources including control, no enzyme activity has been observed however, the growth of *A. tropicalis* was prominent. No activity with raffinose also indicated that the crude dextransucrase preparation of *A. tropicalis* was free of levansucrase as this enzyme can form levan from raffinose **(Andersone *et al.*, 2004)**. This absolute dependence of dextransucrase on sucrose has also been reported by **Robyt (1985)**. However, *Streptococcus* spp. does not require sucrose in the medium and produce dextransucrase constitutively **(Leathers, 2002; Robyt *et al,* 2008).**

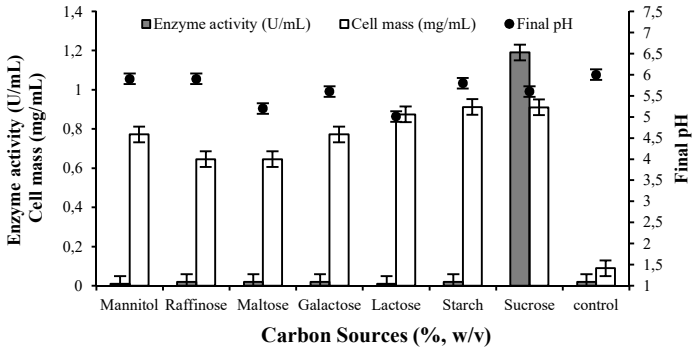

Figure 7 Selection of carbon sources for maximum cell mass and dextransucrase production by *A. tropicalis*

In order to find out the optimum value of sucrose for *A. tropicalis* dextransucrase production, different concentrations (0.5-5.0%, w/v) of sucrose were used in a medium (pH 7.0) containing (%, w/v) peptone 1.0. The results showed that with increase in sucrose concentration (up to 2.5%, w/v) the cell mass as well as dextransucrase production increased (Fig. 8). Dextransucrase activity and cell mass at 2.5% (w/v) were 1.36 U/mL and (0.92 mg/mL, respectively. However, pH of the medium lowered down from 7.0 to 4.20. On further increase in sucrose concentration more than 2.5 (% w/v), the cell mass became constant and enzyme production continuously decreased due substrate inhibition.

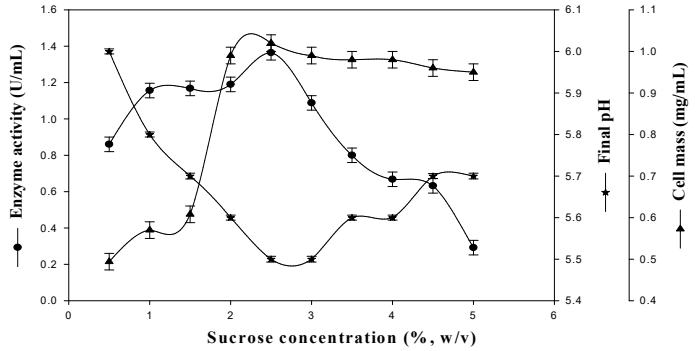

Figure 8 Effect of sucrose concentration on cell mass production and dextransucrase activity of *A. tropicalis*

The study by Patel and coworkers **(Patel *et al.*, 2011)** showed that 5% (w/v) sucrose concentration was optimum for maximum enzyme activity (4.4 U/mL), which corroborated the findings on *L. mesenteroides* NRRL B-640 and *P. pentosaceus* dextransucrase (**Purama and Goyal, 2009; Bhawani and Sunder , 2014**).

Optimization of organic nitrogen sources

Various water soluble organic nitrogen sources were used at a concentration of 1.0% (w/v) in production medium (pH 7.0) (Fig. 9). Media were inoculated with 1% (v/v) seed of *A. tropicalis* and incubated at 25 ºC. Medium which was containing (%, w/v) peptone 1.0 and sucrose 2.5 was considered as control. Among all the nitrogen sources, peptone was found to be the most effective in terms of cell mass (1.03 mg/mL) as well as dextransucrase production (1.36 U/mL). Second best nitrogen source was found to be casein, which showed 0.56 mg/mL cell mass production and 1.20 U/mL enzyme activity (Fig. 9).

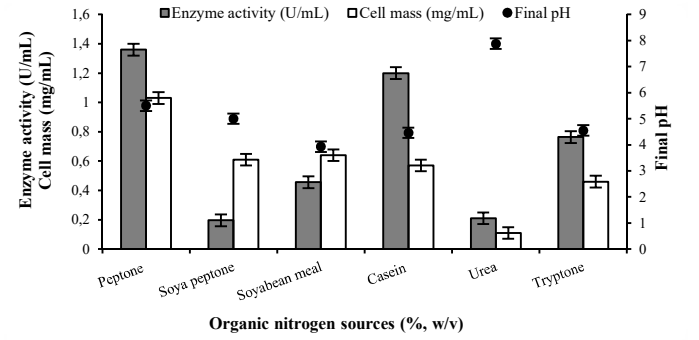

Figure 9 Effect of organic nitrogen source on growth and dextransucrase production by *A. tropicalis*

However, in other nitrogen sources the enzyme production was very poor. Urea was proved to be the most inferior nitrogen source with minimum growth and dextransucrase production. Considering this, concentration of peptone (0.25-3%, w/v) was also varied in the production medium (pH 7.0) containing (%, w/v) sucrose 2.5 in order to find out its optimum value. The 1.5% (w/v) concentration of peptone was found to be optimum for maximum growth (1.05) and dextransucrase production (1.56 U/mL) by *A. tropicalis* (Fig. 10). In previous work also, peptone favored the dextran and dextransucrase production by *L. acidophilus* ST76480.01 **(Rania *et al.*, 2013).**

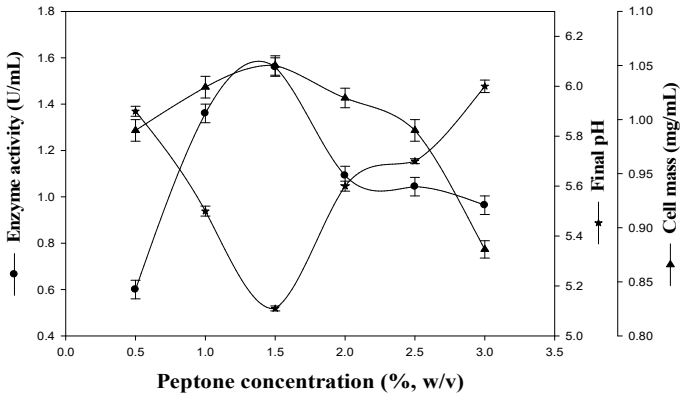

Figure 10 Effect of peptone concentration on cell mass and dextransucrase production by *A. tropicalis*

Optimization of growth supplements

Like any other medium components, addition of growth supplements generally improves the cell mass production because they act as source of carbon, nitrogen and vitamins for bacterial growth. Four different complex materials at a concentration of 1.0% (w/v) were added separately to a medium (pH 7.0) containing (%, w/v) peptone 1.5 and sucrose 2.5. The medium without any growth factor was considered as control. Yeast extract gave maximum growth (1.07 mg/mL) as well as dextransucrase (2.28 U/mL) production by *A. tropicalis* (Fig. 11). Malt extract and meat extract were proved to be most inferior supplements for dextransucrase production. Since, these growth factors are also the source of nitrogen, the production variation may be due to the nitrogen content of the growth supplements.

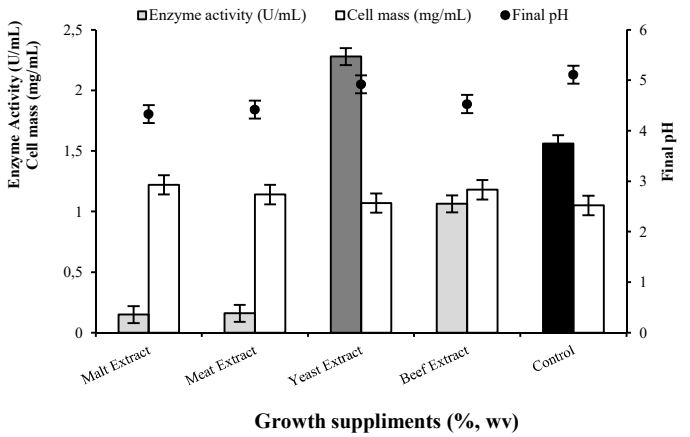

Figure 11 Effect of growth supplements on cell mass and dextransucrase production by *A. tropicalis*

Concentration of yeast extract (0.5-3.0%, w/v) was varied in the medium to find out its optimum value. Maximum growth (1.09 mg/mL) was obtained at 0.5% (w/v) concentration of yeast extract but the maximum dextransucrase (2.93 U/mL) was observed at 1.5% (w/v) (Fig. 12). **Purama and Goyal (2009)** also reported the effect of beef extract (1.5%, w/v) on dextransucrase production from *L. mesenteroides* NRRL B-640 by15%. Whereas, for *L. mesenteroides* T3, combination of beef extract and 1% yeast extract resulted in 4.52 U/mL dextransucrase activity **(Miljkovic *et al.*, 2017).**

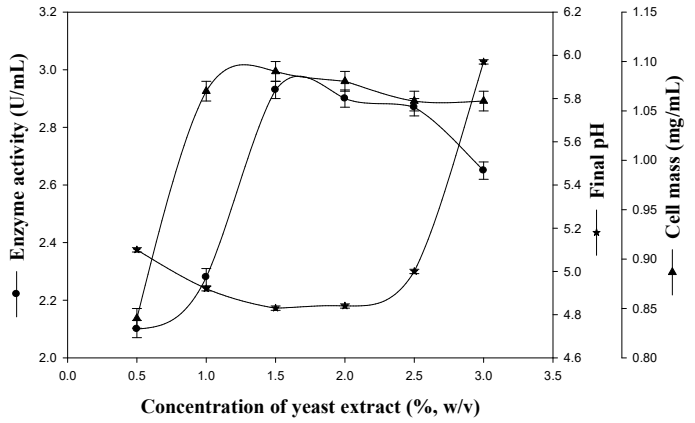

Figure 12 Optimization of yeast extract concentration for maximum growth and dextransucrase production by *A. tropicalis*

Effect of metal ions, additives, inhibitors and chelating agents

Various metal ions, chelating agent, inhibitors and additives were added in the production medium (%, w/v, sucrose 2.5, peptone 1.5 and yeast extract 1.5, pH 7, 25 ºC). A medium without any metal ions, surfactants, inhibitors and additives was considered as control. Most of the metal ions showed negative results except Mn^{2+} which gave dextransucrase activity (3.59 U/mL) more than that in control (2.93 U/mL). Presence of Hg^{2+}, EDTA and Cu^{2+} in the production medium inhibited dextransucrase production (Fig. 13).

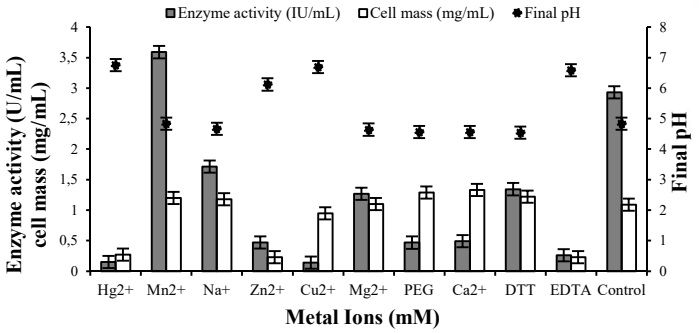

Figure 13 Role of metal ions, additives, inhibitors and chelating agent on the growth and production of dextransucrase by *A. tropicalis*

Considering these results, production medium with Mn^{2+} ($MnSO_4$) was used in every further experiment. In order to optimize the value of $MnSO_4$ in the production medium, concentration was varied from 0.3-3.0 mM (w/v). Maximum growth and (1.4 mg/mL) dextransucrase activity (4.31 U/mL) was found in 1.5 mM $MnSO_4$ (Fig. 14).

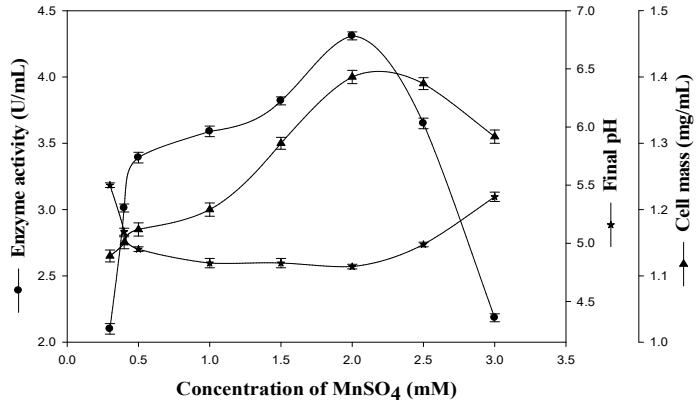

Figure 14 Effect of concentration of $MnSO_4$ on cell mass and dextransucrase production by *A. tropicalis*

Dols and coworkers reported Mn^{2+} to be essential for the dextransucrase production from *L. mesenteroides* NRRL B-1299 **(Dols *et al.*, 1998)**. Bellengier and his coworkers also documented that the addition of Mg^{2+}, Mn^{2+} and amino acids stimulated the growth of most *Leuconostoc* strains **(Bellinger *et al.*, 1997)**. They also stated that Mn^{2+} suppressed the inhibitory effect of aeration on the growth of *L. mesenteroides* UD-23 and suggested its protective role against oxygen toxicity on *L. mesenteroides* UD-23. The positive effect of the 0.005 % MnSO4 on the *L. mesenteroides* NRRL B-640 dextransucrase production was also reported by **Purama and Goyal (2007)**

Effect of inorganic salts

Various inorganic salts (0.5%, w/v) *viz.* $NaHCO_3$, CH_3COONa, Na_2HPO_3, NaH_2PO_3, K_2HPO_3, KH_2PO_3 etc. were added in the production medium to study their effect on the growth and dextransucrase production by *A. tropicalis*. CH_3COONa gave the maximum enzyme activity (4.58 U/mL) with 1.6 mg/mL growth and final pH 4.7(Fig. 15). Activity of dextransucrase of *A. tropicalis* was found to decrease in the presence of phosphate. However, Rodrigues and coworkers reported that the phosphate-enriched medium is known to maintain the pH values above 5.0, in which the enzyme was found to be stable **(Rodrigues *et al.*, 2003)**.

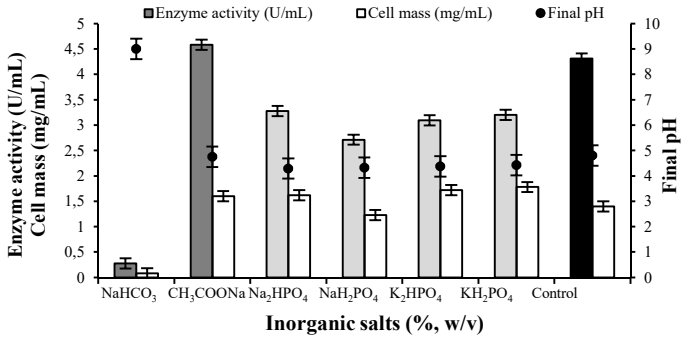

Figure 15 Effect of inorganic salts on growth and dextransucrase production by *A. tropicalis*

The concentration of CH_3COONa in the production medium (pH 7.0) was varied ranging from 0.1-1.75% (w/v). Media were inoculated with *A. tropicalis* 1% (v/v) seed (12 h) and incubated at 25ºC and for 8 h. Since, dextransucrase from *A. tropicalis* showed the maximum activity (4.58 U/mL) with previously used concentration (0.5%, w/v) which was almost similar to the activity (4.58 U/mL) shown with 0.25% (w/v) of CH_3COONa in the production medium, 0.25% (w/v) was used in the production medium for the further experiments to minimize the cost of the medium (Fig. 16).

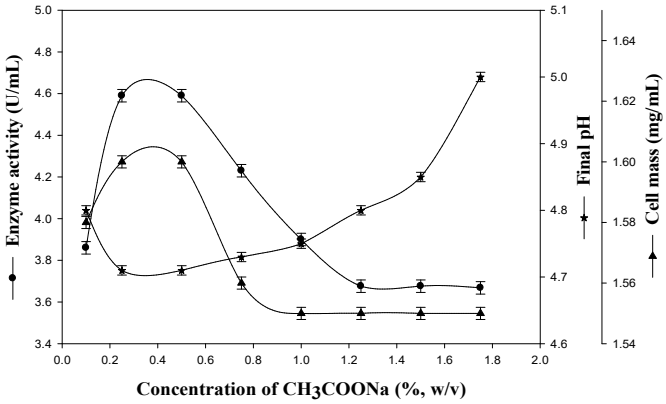

Figure 16 Effect of CH_3COONa concentration on growth and dextransucrase production by *A. tropicalis*

Effect of individual parameters resulted in formation of new medium with the composition; %, w/v, Sucrose 2.5, peptone 1.5, yeast extract 1.5, CH_3COONa 0.25 and $MnSO_4.2H_2O$ 2mM 0.033 (2 mM). All these studies improved the *A. tropicalis* dextransucrase production by 20-fold over the basal medium.

DESIGNING 2^6 TECHNOLOGICAL COMBINATIONS ON OPTIMIZED PRODUCTION PARAMETERS

Technological combinations were designed to obtain the best combination of physicochemical parameters for the maximum production of dextransucrase by *A. tropicalis*. It was interesting to note that for all 64 combinations of factorial experiment designed on physicochemical parameters (data not shown), maximum dextransucrase production (4.87 U/mL) was obtained with 16th combination [pH 7.0, temperature 25 ºC and (%, w/v) sucrose 2.0, peptone 1.0, $MnSO_4.2H_2O$ 0.025, CH_3COONa 0.50)].

This combination showed greater dextransucrase activity (4.87 U/mL) with reduced cell mass production (1.15 mg/mL) than the optimized control medium (4.59 U/mL enzyme and 1.6 mg/mL cell mass) with the composition %, w/v, Sucrose 2.5, peptone 1.5, yeast extract 1.5, $MnSO_4.2H_2O$ 0.033 (2 mM), CH_3COONa 0.25. This experiment improved dextransucrase production by 21.17 folds over the previously selected basal medium (%, w/v, peptone 1.0, meat extract 0.8, yeast extract 0.4, sucrose 2.0, CH_3COONa 0.5, K_2HPO_4 0.2, $MgSO_4.7H_2O$ 0.02, $MnSO_4.2H_2O$ 0.005).

These results revealed that the physicochemical parameters were dependent on each other as they worked better in combination to enhance the dextransucrase activity as compared to the individual optimized parameters. The selected technological combinations also proved to be very satisfactory on economical point of view because, concentration of sucrose, peptone and $MnSO_4.2H_2O$ were reduced. Sawale and coworkers applied response surface methodology (RSM) for enhanced dextransucrase production from strain *L. mesenteroides* UICT/LI18 which showed 5.42 folds increase in dextransucrase activity (8.15 U/ml) over the basal medium (**Sawale *et al.*, 2010b**). Recently, it has been reported that dextransucrase production from *W. confusa* enhanced the enzyme yield from 0.54 U/mL to 2.51 U/mL with 4.6-fold upsurge after the implementation of the Plackett-Burman design **(Zafar *et al.*, 2018).**

CHARACTERIZATION OF CRUDE DEXTRANSUCRASE OF *A. TROPICALIS*

Selection of buffer and optimization of pH

Eight different buffers of varying pH (4.0-10.0) at 0.1 M concentration were used (data not shown) to select buffer of optimum pH for *A. tropicalis* dextransucrase activity. The enzyme was found to work best at pH range of 5.0-5.5 of sodium acetate buffer but maximum activity (4.88 U/mL) was observed with pH 5.5. The alteration in pH optimum may destabilize the structure of the enzyme and the enzyme begins to lose its shape and affinity towards substrate. The optimum pH of 5.2-5.4 have also been reported for dextransucrase of *L. mesenteroides* NRRL B-512(F) **(Goyal *et al.*, 1995)**, *L. mesenteroides* NRRL B-640 **(Purama and Goyal, 2009)** and *L. dextranicum* NRRL B-1146 **(Majumder and Goyal, 2008)**.

The molarity of sodium acetate buffer (pH 5.5) was varied (10-200 mM) to find out optimum ionic strength of the buffer for maximum activity of *A. tropicalis* dextransucrase. The results (Fig. 17) showed maximum enzyme activity (5.38 U/mL) with 25 mM sodium acetate buffer (pH 5.5). Study on *L. mesenteroides* NRRL B-640 dextransucrase reported that 20 mM sodium acetate buffer (pH 5.4) works best for the enzyme assay **(Purama and Goyal, 2008).** Similar results have been reported for *P. pentosaceus* and *L. mesenteroides* strain MTCC 107 dextransucrase where enzymes work best in 20 mM sodium acetate buffer with pH 5.4 and 5.5, respectively **(Patel *et al.*, 2011; Ghai *et al.*, 2015).**

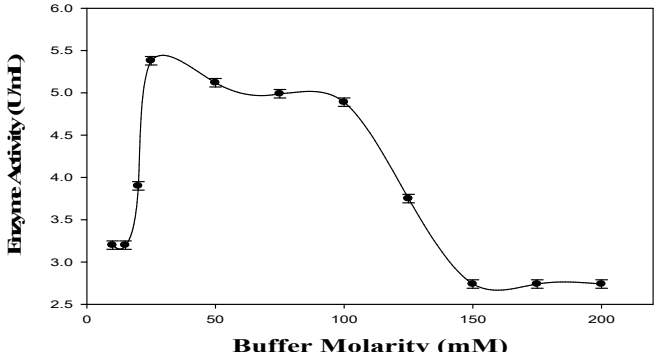

Figure 17 Effect of molarity of sodium acetate buffer on activity of crude dextransucrase of *A. tropicalis*

Optimization of reaction temperature

Dextransucrase of *A. tropicalis* was incubated at different temperature (20-60 °C) with the substrate to find out its optimum value for maximum dextransucrase activity. The 37 ºC reaction temperature was recorded as optimum for the maximum activity (5.38 U/mL). However, with further increase in incubation temperature, dextransucrase activity decreased possibly due to enzyme denaturation (Fig. 18). Optimum temperature for dextransucrase activity from *L. mesenteroides* B-512(F) have been reported in the range of 30-35 °C **(Purama and Goyal, 2005)**. Whereas, 30 °C optimum reaction temperature has been observed for maximum *P. pentosaceus* dextransucrase activity **(Patel *et al,* 2011; Shukla and Goyal, 2014)**.

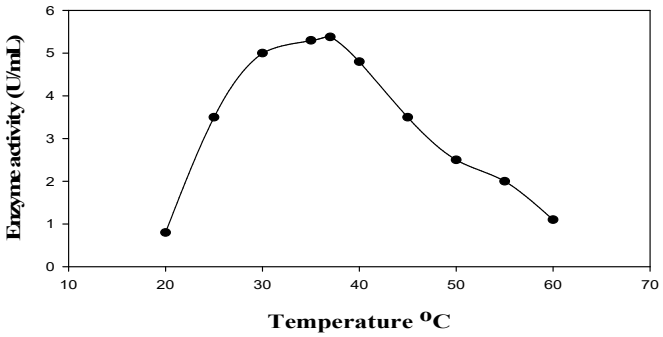

Figure 18 Effect of reaction temperature on activity of crude dextransucrase of *A. tropicalis*

Optimization of Sucrose Concentration

The varying concentrations of sucrose (1-4%, w/v) were used in the reaction mixture to obtain its optimum value for the maximum *A. tropicalis* dextransucrase activity. The maximum enzyme activity (5.39 U/mL) was found with use of 2% (w/v) sucrose in the reaction mixture (Fig. 19). In the previous reports, 10% (w/v) sucrose prepared in acetate buffer (pH 5.2) has been used for the assay of dextransucrase of *L. mesenteroides* NRRL B-512(F) **(Rodrogues *et al.*, 2003; Talita and Sueli, 2010)**. Moreover, Shukla and his co-worker reported 5% sucrose concentration as optimum for maximum activity of dextransucrase (**Shukla and Goyal, 2014**).

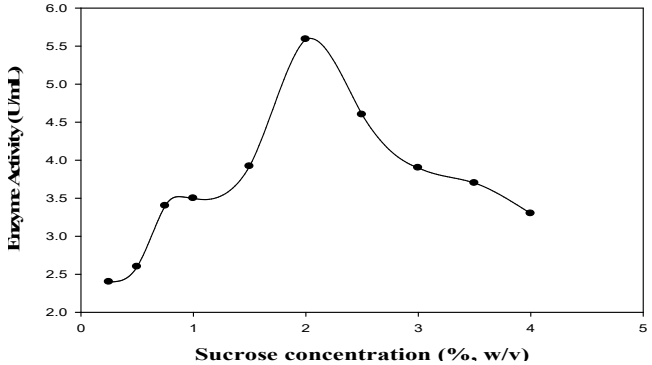

Figure 19 Effect of sucrose concentration on dextransucrase activity of *A. tropicalis*

Effect of metal Ions

The crude *A. tropicalis* dextransucrase activity was assayed in presence of 1 mM concentration of various metal ions (in the form of salts) under optimized conditions. Most of the metal ions inhibited enzyme activity and no activity was found in Pb^+ and Cu^{2+}. Whereas, significant increase in the dextransucrase activity (8.34 U/mL) was observed in the presence of Ca^{2+} (Fig. 20). The increase in enzyme activity in presence of Ca^+ ions suggests that the dextransucrase of *A. tropicalis* requires Ca^{2+} ions for its structural stability and better substrate binding.

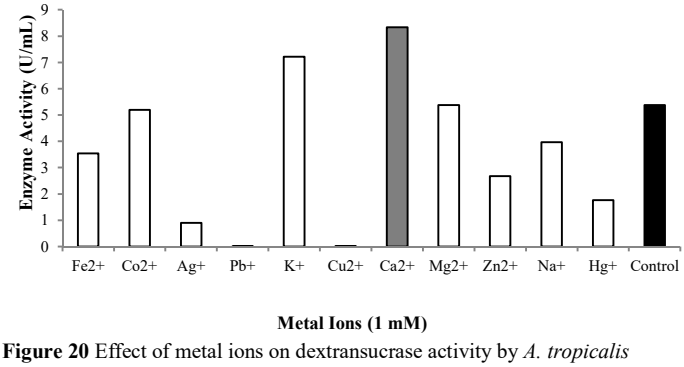

Figure 20 Effect of metal ions on dextransucrase activity by *A. tropicalis*

Considering the significant increase in the enzyme activity in the presence of Ca^{2+} ion, the concentration of Ca^{2+} ion ($CaCl_2$) was varied (0.1-1.5 mM) in the reaction mixture and dextransucrase activity was assayed in every case. Optimum molar concentration of the $CaCl_2$ for maxiumum activity (12.2 U/mL) of dextransucrase was found with 0.2 mM. The results are shown in Fig. 21.

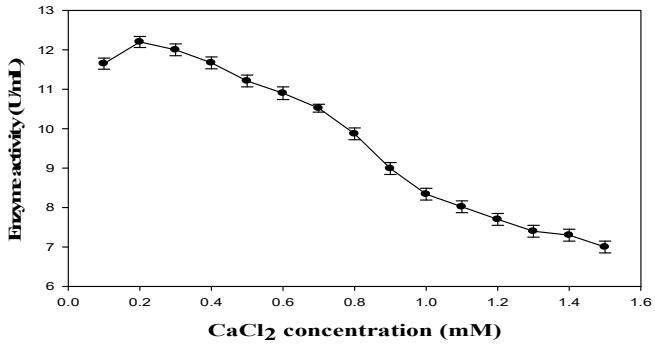

Figure 21 Effect of $CaCl_2$ concentration on dextransucrase activity of *A. tropicalis*

Dextransucrase was reported to have two Ca^{2+} binding sites, activator and inhibitory. Ca^{2+} at the inhibitory site prevents the binding of sucrose **(Miller and Robyt (1986)**. Studies on dextransucrase of *L. mesenteroides* NRRL B-512(F) confirmed that below 1 mM concentration, Ca^{2+} acts as activator of the enzyme by increasing V_{max} and decreasing the K_m for sucrose. However, higher level of Ca^{2+} acts as weak competitive inhibitor **(Miller and Robyt, 1986)**. Ca^{2+} has also been reported for increasing and maintaining the dextransucrase activity by Qader and co-workers **(UI Qader *et al.*, 2007)**.

Determination of K_m and V_{max} values and Thermostability of *A. tropicalis* dextransucrase

Lineweaver-Burk plot was used for determining the K_m and V_{max} values. The K_m and V_{max} values of the crude dextransucrase *A. tropicalis* were found to be 29.86 mM and 7.0 U/mL, respectively (Fig. 22). *K*m and *V*max values of purified *A. tropicalis* dextransucrase have already been reported to be 11.5 mM and 5000 U/mg, respectively **(Nisha and Wamik Azmi, 2014)**. These results showed that purified dextransucrase of *A. tropicalis* had better sucrose binding capacity than the crude enzyme. Dextransucrase of *W. cibaria* also followed Michaelis-Menten kinetics with K_m of 13 mM and V_{max} 27.5 U/mg **(Rao and Goyal, 2013)**.

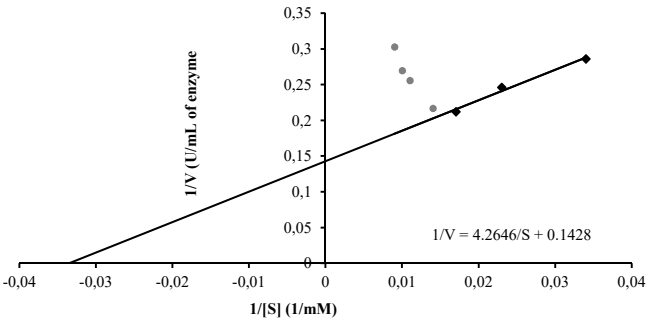

Figure 22 Lineweaver-Burk plot for the crude dextransucrase of *A. tropicalis*

Thermostability of *A. tropicalis* dextransucrase has also been determined at three selective temperatures [4 (storage temp.), 25 (Room temp.), 37 (Body and optimum reaction temp.)]. Whereas, the other two higher temperatures (40 and 50 °C) were selected just to check for how long enzyme retain its activity at higher temperature. The result showed that enzyme was most stable at 4ºC and can be stored for more than a month period. However, The T½ of the dextransucrase at 25 and 37 ºC were found to be 8 and 7 h, respectively (Fig. 23). The T½ of crude dextransucrase at 40 ºC was decreased to 2 h and at 50 ºC enzyme was found to be most unstable.

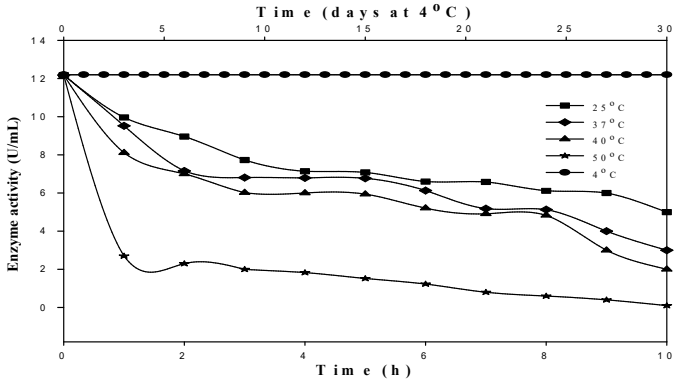

Figure 23 Thermostability of crude dextransucrase of *A. tropicalis* at different temperatures

CONCLUSION

In the present study the newly isolated *A. tropicalis* was found to produce 12.2 U/mL dextransucrase in shake flask culture under optimized production and reaction conditions. This value was far higher than those reported previously by different bacterial strains **(Paul *et al,* 1984; Monchois *et al,* 1997; Funane *et al*, 2000; Fabre *et al,* 2005; Moulis *et al,* 2006; Joucla *et al*, 2006; Iliev and Vasileva, 2012; Rania *et al.,* 2013; Miljkovic *et al.,* 2017, Zafar *et al*., 2018)**. On the other hand, no contamination of extracellular levansucrase was observed during purification process. Absence of levansucrase was confirmed by dextranase treatment and Periodic Acid Schiff (PAS) staining of non-denaturing SDS-PAGE gel (Purama and Goyal 2008; Nisha and Azmi, 2014). This study opens a new horizon to study dextransucrase from novel isolate *A. tropicalis* with short production time and significant activity. Therefor this strain can act as promising candidate commercially for food applications.

Acknowledgements: The authors gratefully acknowledge the financial support by the UGC (University Grant Commission) and Himachal Pradesh University, Summer Hill, Shimla, India, for providing experimental facilities.

REFERENCES

Andersone I., Auzina L., Vigants A., Mutere O. & Zikmanis P. (2004). Formation of Levan from Raffinose by Levansucrase of *Zymomonas mobilis. Eng. Life. Sci., 4* (1), 56-59. https://doi.org/10.1002/elsc.200400006.

Bailey R.W. & Oxford A.E. (1958). Pre-requisites for dextran production by *Streptococcus bovis. Nature, 182*: 185-186. https://doi.org/10.1038/182185a0.

Barker, P.E. & Ajongwhen, N.J. (1991). The production of the enzyme dextransucrase using non-aerated fermentation techniques. *Biotech. Bioeng., 37*, 703-707. https://doi.org/10.1002/bit.260370803.

Bellinger, P., Richard, J. & Foucaud, C. (1997). Nutritional requirements of *L. mesenteroides* subsp. *mesenteroides* and subsp. *dextranicum* for growth in milk. *J. dairy Res., 64*, 95-103. https://doi.org/10.1017/s0022029996001902.

Bhavani A.L. & Sundar S.K. (2014). Optimization of various parameters for enhancement of dextransucrase production by Lactic acid bacteria of the cocci group. *Int. J. Curr. Microbiol. App. Sci., 3*(11), 849-857.

Cortezi, M., Monti, R. & Contiero, J. (2005). Temperature effect on dextransucrase production by *Leuconostoc mesenteroides* FT 045 B isolated from Alcohol and Sugar Mill Plant. *Af. J. Biotechnol., 4*, 279-285.

Dols, M., Remaud, M.S., Willolt, R., Vignon, M. & Monson, P. (1998). Characterization of the different dextransucrase activities excreted in glucose, fructose or sucrose medium by *L. mesenteroides* NRRL B1299. *Applied Environ. Microbiol. 64*, 298-302.

Fabre, E., Bozonnet. S., Arcache, A., Willemot, R.M., Vignon, M., Monsan, P. & Remaud-Simeon, M. (2005). Role of the two catalytic domains of DSR-E dextransucrase and their involvement in the formation of highly alpha 1,2 branched dextran. *J. Bacteriol., 187*, 296-303. https://doi.org/10.1128/jb.187.1.296-303.2005.

Funane, K., Mizuno, K., Takahara, H. & Kobayashi, M. (2000). Gene encoding a dextransucrase-like protein in *Leuconostoc mesenteroides* NRRL B-512F. *Biosci. Biotechnol. Biochem. 64*, 29-38. https://doi.org/10.1271/bbb.64.29.

Ghai K., Batta S.K., Kocher G.S., Kocher G.S. & Gupta A.K. (2015). Production, Partial Purification and Kinetic Characterization of Dextransucrase from *Leuconostoc mesenteroides* in Relation to Dextran Problem in Sugarcane. *Sugar Tech. 17*(2),162-172. https://doi.org/10.1007/s12355-013-0297-7.

Goyal, A., Nigam, M. & Katiyar, S.S. (1995). Optimal conditions for production of dextransucrase from *L. mesenteroides* NRRL B-512F and its properties. *J. Basic Microbiol. 35*, 375-384. https://doi.org/10.1002/jobm.3620350604.

Honorato, T.L. & Rodrigues, S. (2010). Dextransucrase Stability in Cashew Apple Juice. *Food Bioprocess Technol. 3,* 105-110. https://doi.org/10.1007/s11947-008-0053-2.

Hucker, G.J. & Pederson, C.S. (1930). Studies on coccaceae XVI.Genus *Leuconostoc*. *N. Y. Agr. Expt. Sta. Tech. Bull*. *167*, 3-8.

Iliev & Vasileva (2012). Enzymatic production of glucooligosaccharides using dextransucrases from strains *Leuconostoc mesenteroides* Lm 22 and *Leuconostoc mesenteroides* Lm 28 *J. BioSci. Biotech. 1*(1), 51-56.

Jeans, A., Haynes, W.C., WiLham, C.A., Rankin, J.C., Melvin, E.H., Austin, M.J., Cluskey, J.E., Fisher, B.E., Tsuchiya, H.M. & Rist, C.E. (1954). Characterisation and Classification of Dextrans from Ninety-Six Strains of Bacteria. *J. Am. Chem. Soc. 76*, 5041-5052. https://doi.org/10.1021/ja01649a011.

Hashem A.M., Refaei M.A.E., Gebril H.M. & Fattah A.F.A. (2012). Immobilization of *Leuconostoc-paramesenteroides* dextransucrase enzyme and characterization of its enzyme properties. *J. Basic Appl. Sci. 8*: 344-352. https://doi.org/10.6000/1927-5129.2012.08.02.16.

Joucla, G., Pizzut-Serin, S., Monsan, P. & Remaud-Simeon, M. (2006). Construction of a fully active truncated alternansucrase partially deleted of its carboxy-terminal domain. *FEBS Lett. 580*, 763-768. doi.org/10.1016/j.febslet.2006.01.001.

Koepsell, H.J. & Tsuchiya, H.M. (1952). Enzymatic synthesis of dextran. *J. Bact. 63*, 293-295.

Lazic M.L., Veljkovic V.B., Vucetic J.I & Vrvic M.M. (1993). Effect of pH and aeration on dextran production by *Leuconostoc mesenteroides*. *Enz. Microb. Technol. 15*(4), 334-338. https://doi.org/10.1016/0141-0229(93)90160-4.

Leathers, T.D. (2002). Dextran. (Vandamme, E.J., De Baets, S. & Steinbuchel, A., Wiley, V.C.H., Weinheim, eds). *Biopolymers. 5*, 299-321. https://doi.org/10.1002/3527600035.bpol5012.

Majumder, A. & Goyal, A. (2008). Enhanced production of exocellular glucansucrase from Leuconostoc dextranicum NRRL B-1146 using response surface method. *Biores. Technol*. *99*, 3685-3691. https://doi.org/10.1016/j.biortech.2007.07.027.

Miljkovic M.G., Sladana Z., Davidovic, Kralj S., Slavica S., Marinkovic R., Mirjana D., Stojanovic R., Suzana I. & Brankovic D. (2017). Characterization of dextransucrase from *Leuconostoc mesenteroides* T3, water kefir grains isolate. *Hem. Ind. 71*(4), 351-360. https://doi.org/10.2298/hemind160421046m.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent of determination of reducing sugar. *Anal. Chem. 31*, 426-428. https://doi.org/10.1021/ac60147a030.

Miller, A.W. & Robyt, J.F. (1986). Functional molecular size and structure of dextransucrase by radiation inactivation and gel electrophoresis. *Biochim. Biophys. Acta. 870*, 198-203. https://doi.org/10.1016/0167-4838(86)90222-0.

Monchois, V., Remaud-Simeon, M., Russell, R.R., Monsan, P. & Willemot, R.M. (1997) Characterization of *Leuconostoc mesenteroides* NRRL B-512F dextransucrase (DSRS) and identification of amino-acid residues playing a key role in enzyme activity. *Appl. Microbiol. Biotechnol*. *48*, 465-472. https://doi.org/10.1007/s002530051081.

Moulis. C., Arcache, A., Escalier, P.C., Rinaudo, M., Monsan, P., Remaud-Simeon, M. & Potocki-Veronese, G. (2006). High-level production and purifcation of fully active recombinant dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. *FEMS Microbiol. Lett. 261*, 203-210. https://doi.org/10.1111/j.1574-6968.2006.00347.x.

Naessens M., Cerdobbel A., Soetaert W. & Vandamme E.J. (2005). *Leuconostoc* dextransucrase and dextran: production, properties and applications. *J. Chem. Technol. Biotechnol. 80*: 845-860. https://doi.org/10.1002/jctb.1322.

Nisha, & Azmi, W. (2014). Purification and Characterization of a Novel High Molecular Weight Dextransucrase from *Acetobacter tropicalis*. *J. Advance microb. 1*(3), 184-196.

Patel, S. & Goyal, A. (2010). Isolation, characterization and mutagenesis of exopolysaccharide synthesizing new strains of lactic acid bacteria. *I. J. Microbiol. 8*(1), 3-4. https://doi.org/10.5580/1bbb.

Patel, S., Kothari, D. & Goyal, A. (2011). Purification and characterization of an extracellular dextransucrase from *Pediococcus pentosaceus* isolated from the soil of north east India. *Food. Technol. Biotechnol. 49*(3), 297-303.

Paul, F., Auriol, D., Oriol, E. & Monsan, P. (1984). Production and purification of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512(F). *Ann. NY Acad. Sci. 434*, 267-270. https://doi.org/10.1111/j.1749-6632.1984.tb29841.x.

Purama R.K. & Goyal A. (2005). Dextransucrase production by *Leuconostoc mesenteroides*. *Ind. J. Microbiol. 45*: 89-101.

Purama R. & Goyal A. (2007). Effect of Nutrients by One Variable At A Time (OVAT) Approach on the Dextransucrase Production from Leuconostoc mesenteroides NRRL B-640. *The Internet J. Microbiol*. *5*(1), 1-8. https://doi.org/10.5580/1bc.

Purama, R.K. & Goyal, A. (2008). Identification, effective purification and functional characterization of dextransucrase from *Leuconostoc mesenteroides* NRRL B-640. *Biores. Technol*. *99*(9), 3635-3642. https://doi.org/10.1016/j.biortech.2007.07.044.

Purama, R.K. & Goyal. A. (2009). Optimization of conditions of *Leuconostoc mesenteroides* NRRL B-640 for production of dextransucrase and its assay. *J. Food Biochem. 33*, 218-231. https://doi.org/10.1111/j.1745-4514.2009.00219.x.

Rao, T.J. & Goyal, A. (2013). Purification, optimization of assay, and stability studies of dextransucrase isolated from *Weissella cibaria* JAG8. *Prep. Biochem. Biotechnol. 4*, 329-341. https://doi.org/10.1080/10826068.2012.737400.

Robyt, J.F. (1986). Dextran. (Mark HF, Bikales NM, Overberger CG, Menges G eds). In: Encyclopedia of polymer science and technology. *15*, 752–767.

Robyt, J.F. & Walseth, T.F. (1979) Production, purification, and properties of dextransucrase from *Leuconostoc mesenteroides* B-512 F. *Carbohyd. Res. 68*, 95-11. https://doi.org/10.1016/s0008-6215(00)84059-8.

Robyt, J.F., Yoon, S.H. & Mukerjea, R. (2008). Dextransucrase and the mechanism for dextran biosynthesis. *Carbohydr. Res. 343*, 3039-3048. https://doi.org/10.1016/j.carres.2008.09.012.

Rodrigues, S., Lona, L.M.F. and Franco, T.T. (2003) Effect of phosphate concentration on the production of dextransucrase by *Leuconostoc mesenteroides* NRRL B512F. *Bioprocess Biosyst. Eng. 26*, 57-62. https://doi.org/10.1007/s00449-003-0330-4.

Santos, M., Teixeira, A. & Rodrigues, A. (2000). Production of dextransucrase, dextran and fructose from sucrose using *L. mesenteroides* NRRL B512(f). *Biochem. Eng. J. 4*, 177-188. https://doi.org/10.1016/s1369-703x(99)00047-9.

Sarwat, F., Ul-Qader, S.A., Aman, A. & Ahmed, N. (2008). Production & Characterization of a Unique Dextran from an Indigenous *Leuconostoc mesenteroides* CMG713. *Int. J. Biol. Sci. 4*(6), 379-386. https://doi.org/10.7150/ijbs.4.379.

Shukla R. & Goyal A. (2014). Purified dextransucrase from *Pediococcus pentosaceus* CRAG3 as food additive. *In. J. Exp. Biol. 52,* 1036-1044.

Shukla R., Iliev I. & Goyal A. (2010). Purification and Characterization of Dextransucrase from *L. Mesenteroides* NRRL B-1149, *Biotechnol. Biotechnol. Eq.*, *24*(1), 576-580. https://doi.org/10.1080/13102818.2010.10817900.

Rania M.A., Abedin, Aliaa M., El-Borai, Shall M.A., Samy A. & El-Assar (2013). Optimization and Statistical Evaluation of Medium Components Affecting Dextran and Dextransucrase Production by *Lactobacillus acidophilus* ST76480.01. *Life Science. J. 10*(1), 1746-1753.

Santos M., Teixeira J. & Rodrigues A. (2000). Production of dextransucrase, dextran and fructose from sucrose using *Leuconostoc mesenteroides* NRRL B512 (F). *Biochem. Eng. J. 4*: 177-188. https://doi.org/10.1016/s1369-703x(99)00047-9.

Sawale, S.D. & Lele, S.S. (2010_a). Increased Dextransucrase Production by Response Surface Methodology from *Leuconostoc* Species; Isolated from Fermented *Idli* Batter. *Global J. Biotechnol. Biochem. 4*(2), 160-167.

Sawale, S.D. & Lele, S.S. (2010_b) Statistical optimization of media for dextran production by *Leuconostoc* sp., isolated from fermented *idli* batter. *Food Sci. Biotechnol. 19,* 471-478. https://doi.org/10.1007/s10068-010-0066-2.

Talita, L.H. & Sueli, R. (2010). Dextransucrase Stability in Cashew Apple Juice. *Food Biopros. Technol. 3*, 105-110. https://doi.org/10.1007/s11947-008-0053-2.

Ul Qader, S.A., Iqbal, L., Rizvi, H.A. & Zuberi, R. (2001). Production of dextran from sucrose by a newly isolated strain of *Leuconostoc mesenteroides* (PCSIR-3) with reference to *Leuconostoc mesenteroides* NRRL-B512F. *Biotechnol. Appl. Biochem*. *34*, 93-97. https://doi.org/10.1042/ba20010020.

Ul Qader, S.A., Iqbal, L., Aman, A., Shireen, E. & Azhar, A. (2005). Production of dextran by newly isolated strain of *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9. *Turk. J. Biochem. 31*, 21-26.

Ul Qader, S.A., Amer, A., Sayed, N., Bano, S. & Azhar, A. (2007). Characterization of dextransucrase immobilized on calcium-alginate beads from *Leuconostoc mesenteroides* PCSIR-4. *Ital. J. Biochem. 56*, 158-62.

Vidal, R.F., Martınez, A., Moulis, C., Escalier, P., Morel, S., Remaud-Simeon, M. & Monsan, P. (2011). A novel dextransucrase is produced by *Leuconostoc citreum* strain B/110-1-2: an isolate used for the industrial production of dextran and dextran derivatives. *J. Ind. Microbiol. Biotechnol. 38*(9), 1499-506. https://doi.org/10.1007/s10295-010-0936-x.

Vuillemina M., Grimauda F., Claveriea M., Rolland-Sabatéb A., Garnierb C., Lucasd P., Monsane P., Dols-Lafargued M., Remaud-Siméona N. & Moulisa C. (2018). A dextran with unique rheological properties produced by the dextransucrase from *Oenococcus kitaharae* DSM 17330. *Carbo. Poly. 179,* 10–18. https://doi.org/10.1016/j.carbpol.2017.09.056.

Yamaner C.I., Sezen I.Y. & Tanriseven A. (2010). Selection of Psychrotrophic *Leuconostoc* spp. from native fruits, and studies on their dextransucrases. *Food Sci. Biotechnol. 19*(1): 175-184. https://doi.org/10.1007/s10068-010-0024-z.

Zafar S.B., Asif T., Ul Qader S.A. & Aman A. (2018). Enhanced biosynthesis of dextransucrase: A multivariate approach to produce a glucosyltransferase for biocatalysis of sucrose into dextran. *Int. J. Biol. Macromol. 115*, 776-785. https://doi.org/10.1016/j.ijbiomac.2018.04.089.

Zhang, H.B., Hu, Y.J., Zhu, C.B., Zhu, B.Q. & Wang, Y.J. (2008). Cloning, sequencing and expression of a dextransucrase gene (*dex*YG) from *Leuconostoc mesenteroides* LM-0326. *Biotechnol. Lett. 30*, 1441-1446. https://doi.org/10.1007/s10529-008-9711-8.