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Application of Cold-Active Dextranase in Dextran Degradation and Isomaltotriose Synthesis by Micro-Reaction Technology

A.R. Doaa Mahmoud and A. wafaa Helmy

Department of Chemistry of Natural and Microbial Products, National Research Centre, Cairo, Egypt.

Abstract: The object of increasing awareness about the need for improving and reducing energy consumption forced us to isolate more extremozymes from cold loving microorganisms which are able to function with reduced energy requirement. Therefore isolation of psychrophilic *Streptomyces anulatus* that capable of utilizing different forms of soluble and insoluble dextrans at 4°C was undertaken from water samples of Qaron Lake. The isolated strain produced cold-active dextranase which retained 100 % of relative activity at 20°Cand 40 % at 4°C. Dextranase activity was uninhibited by excess dextran concentrations and remained unaffected by changes in temperature (5, 20, 30°C) recording advantage in being applicable in moderate or sever dextran problems in sugar processing. Cold active dextranase of the isolated *Streptomyces anultus* classified as extracellular endodextranase because isomaltotriose was the only product of dextran degradation. Immobilization of the enzyme on hydroxyapatite in micro reactor led to production of 0.7mg isomaltotriose/ml/min. compared with macro reactor 0.45 mg isomaltotriose /ml/min. This rapid mass transfer is the key advantage of micro reaction technology. In this respect this direction of research may be of interest in the innovative application of cold-active dextranase in synthesis of pure isomaltotriose and overcoming dextran problems at lower temperature especially in the crystallization process.

Key words: Cold-active dextranase · Dextran degradation · Isomaltotriose synthesis and micro-reaction technology

INTRODUCTION

The majority of interest in temperature-sensitive extremozymes have focused on the products of thermophilic or hyperthermophilic species. Although understanding the basis of thermostability carries significant biotechnological potential, thermophilic environments are not abundant (Eichler, 2001). In contrast, cold habitats are widespread. low temperature environments prevail in fresh and marine waters, polar and high alpine soils and water glaciers, plants and animals. Moreover the major man made cold environments are refrigerators and freezers and freezers of industrial and domestic food storage. Oceans represent 7% of earth's surface and 90% by volume, which are at 5°C or colder (Satyanaragaue *et al.*, 2005). Cold environments, present proteins with a number of physical challenges which could find use in numerous applications (Russel, 2000).

More recently, enzymes from Psychrophiles have become interesting for industrial application, because of ongoing efforts to decrease energy consumption. Several food processing applications would benefit from the availability of low temperature enzymes (Bertus, 2003).To date a number of cold-active enzymes from psychrophilic microorganisms have been reported such as, alcohol dehydrogenase (Takayuki *et al.*, 2007), Cellulase (Garsoux et al 2004), alkaline serine protease (Son and kim, 2003), protein-tyrosine-phosphatase (Tsuruta and Aizono, 2003), alkaline phosphatase (Murakawa *et al.*, 2002), Citrate synthase (Gerike *et al.,* 2001), phosphoglycerate kinase (Mandelman *et al*., 2001) and pectinolytic enzymes (Nakagawa *et al*., 2002). In the development of enzyme process, the use of immobilized enzymes is preferable. Several methods are available to immobilize enzymes on supports in conventional reaction apparatus and these techniques have also been applied to immobilize enzymes within a micro space in a technology known as micro-reaction technology (Miyazaki and Maeda 2006). Micro reaction technology is an interdisciplinary field combining science and engineering. This technology expected to be a new and promising in the fields of chemistry, enzymology

Corresponding Author: A.R. Doaa Mahmoud, Department of Chemistry of Natural and Microbial Products, National Research Centre, Cairo, Egypt.

and biotechnology (Watts and Haswell 2004 and Krenkova and Foret, 2005).The key advantage of this technology is the rapid mass transfer and use reaction apparatus with small dimensions. Furthermore, the micro reaction (micro channel reaction) systems take advantage of transporting and manipulating of micr- or nano- amounts of fluid through a micro channel to enable the use of drastically reduced volumes of reactant solutions and they offer performance of high efficiency and repeatability (Miyazak and Maeda, 2006).

In Sugar production, dextrans are undesirable compounds produced by *Leuconostoc mesedteroides* which are able to utilize high percentages of the sugar present in juices in a short time period. This implies important losses in sucrose. Dextran contaminated the industrial process and cause various problems such as: an increase of juice viscosity which produces blockage in the process line, pumps and filters, decrease in the efficiency and output of crystallization and sucrose losses to molasses. (Clarke *et al.,* 1997 and Cerutti de Guglielmone *et al.,* 2000). Many investigations suggested a minimization of dextran levels in the sugar factory by different methods even with the chemical additions. More recent approaches propose the application of dextranase enzyme for the removal of preformed dextran. However, theses approaches were uneconomical due to high price (Jimēnez, 2005) or not practical because temperature is known to have a critical effect on the activity of dextranases (Egglestone *et al.*, 2006 and Egglestone and Monge, 2005).

The most damaging effects of elevated dextran concentrations in a technical sucrose solution are foreseen in the crystallization process. Dextran slow down the crystallization rate or even inhibit crystallization. The decrease in crystallization temperature is desirable regarding energy aspects but is not applicable in occurrence of dextran because the influence of dextran increases strongly with the decrease of temperature (Abdel Rahman, 2008).

Consequently, our innovative idea for isolation of psychrophilic microorganisms capable of producing cold active dextranase has been stimulated by the realization that such cold-active dextranase may offer novel opportunities for improvement of sugar industry.

The present investigation is the first report concerning with the isolation or production of cold-active dextranase and the synthesis of isomaltotriose by micro reaction technology.

MATERIAL AND METHODS

Materials:

Soluble dextran with different molecular weights (BDH), Sephadex G-200, LH 20 and DEAE Sephadex-50 (Sigma), glucose, isomaltose, isomaltotriose, maltotriose (Fluka), hydroxyapatite in micro particle size (kindly provided by Biomaterial Department in National Research Center). Other chemicals are of analytical grads.

Water Samples:

Water samples were collected in winter from Qaron Lake which is located in Fayyoum depression in Egypt, Qaron Lake is the essential source for extracting some economic salts such as sodium sulfate and sodium chloride. Five samples were collected from different locations in the Lake. The pHs of all samples were 7.0 -7.8 and the temperature of water samples were about 20°C.

Isolation of Psychrophilic Dextranase Producing Microorganisms:

A screening method was used for detection of dextranase-producing microorganisms from water samples of Qaron lake at different temperatures (4-37°C).The method involved two steps of primary and secondary screening using agar medium and liquid medium for selection. The agar medium containing(W/V): 1.0% blue dextran, 0.2% yeast extract, 2.5% agar dissolved in two times diluted lake water(LW).The liquid medium containing (W/V): 1.0% dextran with average molecular weight(40.000, 170.000, 240.000) unless otherwise stated, 0.2 % yeast extract and dissolved in two times diluted lake water(LW).

Enzyme Activity Measurements:

Two methods for enzyme activity measurements, were used according to a modified method of Wang *et al.* (2004). The first method for soluble dextran, in which the reaction mixture contained, in a final volume of 2.0 ml, 50 mM sodium acetate buffer (pH 5.4), 10 mM dextran (M. wt, 40.000) = 2% for the standard assay unless otherwise specified. Incubation was carried out for 1h at 20°C unless otherwise specified. The enzyme final concentration used is

0.0035 mg/ml. The used enzyme concentration is chosen to make it economically approximately equivalent to the nearest priced "non-concentrated" dextranase applied in sugar factories as reported by Egglestone and Monge (2005) unless other wise specified. The reducing sugar librated was assayed using a colorimetric method of Nelson (1944) and Somogyi (1952). One unit (U) of dextranase activity is defined as the amount of enzyme that catalyzes the formation of 1µmol isomaltotriose measured as maltotriose per minute. The second method: for insoluble dextran. The reaction mixture contained, in a final volume of 3.0 ml, 50 mM sodium acetate buffer (pH 5.4). 0.3 % sephadex G-200 or DEAE-sephadex G 50.Incubation was carried out for 24h at 20°C and unless otherwise specified the enzyme with final concentration 0.0035 mg/ml containing 0.02% sodium azide. The activity for sephadex assay, calculated by the equation:

$$Activity\ \frac{Total\ height\ of\ gel - remaining\ height\ of\ gel}{Total\ height\ of\ gel} \times 100$$

Enzyme Preparation:

To known volume of culture filtrate, the ammonium sulfate was added to 80 % saturation. After standing overnight at 4°C. the precipitation formed was collected by centrifugation at 4°C.The precipitate was dialyzed against assay buffer to remove ammonium sulfate. The dialyzed enzyme was then lyophilized and protein amounts were determined by the method of Lowery *et al*. (1951).

Immobilization of Dextranase to Hydroxyapatite:

Five g of hydroxyapatite(micro particle size) used as carrier and 50 ml of sodium acetate buffer solution having a concentration of 0.05 M and a pH value of 5.4 were added to a mixture of 50 mg albumin with 50 mg of dextranase(albumin used as stabilizer). The resulting product was cooled down to 4°C and was vigorously stirred. While maintaining that temperature, 125 µl of a 0.2% aqueous solution of glutaraldehyde was added drop wise under agitation, followed by additional stirring for 5h. The reaction product was collected by filtration and was washed three times with 100 ml of the aforementioned buffer solution to obtain immobilized hydroxyapatite in un-dried state. Freeze-drying yielded 5g of powdery dextranase immobilized hydroxyapatite.

Chromatographic Analysis of Dextran Hydrolytic Products:

Hydrolytic products from all kinds of dextran used either soluble dextran such as dextran with different molecular weights or insoluble dextran such as sephadex G-200 and DEAE-sephadex G 50 were analyzed by either paper chromatography on whatman No.1 or by TLC using aluminum-backed silica gel.60 sheets(Merck K daA, Germany). The reaction mixtures at the end of incubation time were boiled for 3min to stop the reaction. Chromatographic development was carried out with a solvent system of n-butanol: acetone: water (4:5:1) and detected by spraying with aniline hydrogen phthalate.

Temperature Dependence of Activity:

Temperature dependence of cold-active dextranase was measured by using salted out dextranase with 80% saturation of ammonium sulfate. And substrates dextran (40.000 M. wt) with different concentrations in sodium acetate buffer pH 5.4. The pH chosen to be similar to that applied in the sugar factory.

RESULTS AND DISCUSSION

Chemical Analysis of Qaron Lake Water:

Chemical analysis of water samples was kindly carried out by the Egyptian salts and minerals company (EMISAL).The results of the chemical compositions are represented in Table (1).

Isolation and Growth Characteristics:

A total of eight strains isolated from the different locations of Qaron Lake, after incubation at 4°C - 37°C on blue-dextran agar medium produced clear halos with different diameters. Of the eight isolates, three strains possessed optimal growth temperature at 15°C and below. Most of the remaining strains grew optimally between 20-30°C while no strains

Table 1: The chemical compositions of Qaron lake water.

Elements	lake water´ concentration (g/L)
Mg	6.3
Na	9.2
K	0.65
Ca	0.84
B	0.038
Mn	0.001
Cu	0.002
Zn	0.001
Fe	0.0004
SO4	6.9
Br	0.8
CO3	0.06
HCO3	0.10

were able to grow at 37°C.The positive isolate produced large clear halos in blue dextran was further checked for dextranase production in liquid medium and identified on the basis of phenotypic characterization in Micro Analytical Center of Faculty of Science, Cairo University in Egypt to be a *Streptomyces anulatus*. The definition for psychrophilic that given by Morita (1975) is the psychrophilic microorganisms requiring growth temperature of 15°C or lower and maximum growth temperature of below 20°C. In accordance, the isolated *Streptomyces anulatuse* of Qaron lake which have optimum growth temperature 4°C (in refrigerator) is therefore psychrophilic.

Lake water requirements of the strain was tested by comparing growth of *Streptomyces anulatus* in lake water broth (liquid medium of isolation) and fresh water by adding most of the metals found in the lake water as determined by chemical analysis of Egyptian salts and minerals company(EMISAL) with the same proportions. It is well documented that Qaron lake contains unique collection of flora that goes back to about 40 million years. The isolated *Streptomyces* preferred the natural lake water media for growth and is therefore supposed to be of not terrestrial origin. In contrast, members of the genus Streptomyces are ubiquitous in terrestrial, it is well documented that Qaron Lake contains unique collection of flora that goes back to about 40 million years. In contrast, members of the genus *Streptomyces* are ubiquitous in terrestrial and freshwater habitats and are also distributed in the world oceans, they are able to propagate and to metabolize under marine conditions, but the vas majority are not dependent on seawater media for growth and are therefore supposed to be of terrestrial origin (Das *et al.,* 2006).

Dextran as a Carbon Source:

As mentioned before, *Streptomyces anulatus* was the most potent of the eight isolates that produced high cold-active dextranase, therefore it was selected for the study. The main research goal of this part of the study was to determine the best type of dextran as a sole carbon source for high growth yield and production of cold-active dextranase. This was carried out by culturing the selected *Streptomyces aulatus* in 250 ml Erlenmeyer flasks containing 100 ml culture medium at 4°°C for an incubation period of 1 week using the static culture technique. The following dextrans were used: dextran with different molecular weights, 40.000, 170.000, 240.000 and insoluble dextrans such as: sephadex G-200, DEAE-sephadex G50 and sephadex-LH 20. During the growth of the isolated *Streptomyces*, the concentration of reducing groups in all dextrans containing medium reach a peak of 17-20µ mol /ml after 4 days and declined at the end of incubation period after one week to approximately zero. This is may be due to the explanation that given by Arnold *et al.* (1998) in a results coincide with the present study, this time course suggested a transient accumulation of

oligosaccharide products, most of which were subsequently taken up by the cells. The relatively high yields of cells and the small residual concentration of reducing sugars in the culture medium support this conclusion.

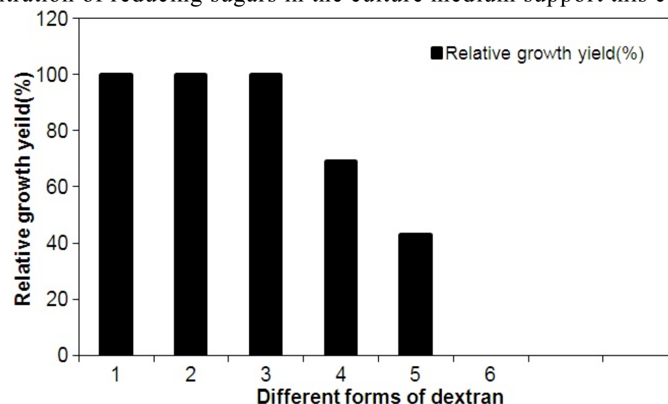

Fig. 1: Effect of different forms of dextran on relative growth yield.
1=Dextran (M. wt 40.000), 2 Dextran (M. wt 170.000), 3= Dextran (M. wt 240.000), 4=DEAE sephadex G50, 5=Sephadex G-200, 6=Sephadex LH 20.

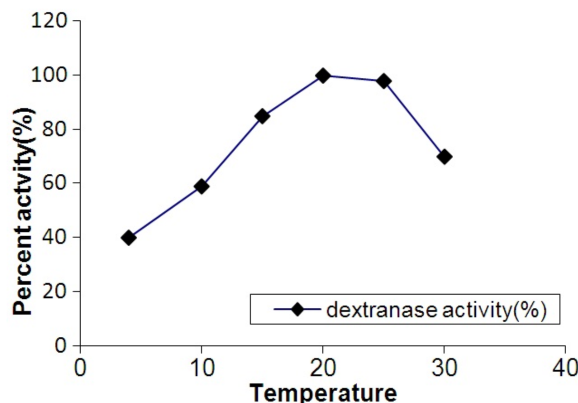

Fig. 2: Effect of temperature on dextranase.

The results in Fig. (1) indicate that most dextrans used supported growth of *Streptomyces anulatus*, the increments in growth yield for soluble dextrans with average molecular weights were 100% as compared to insoluble dextrans. The relative growth for DEAE-sephadex G50, sephadex G-200 and sephadex-LH 20 were 69%, 43%, 0% respectively as compared to soluble dextrans. The result indicated that *Streptomyces anulatus* of Qaron Lake was unable to utilize sephadex- LH 20 as a sole carbon source and preferred soluble dextrans.

Other microorganisms such as *Streptococcus sobinus, Bacteroids oralis, Bacillus circulans, Arthrobacter globiforms, Lipomyces lipofer, Penicillium funiculosm, Aspergillus carneus, Chatomium gracile* and *Sporotix schencki* are capable of utilizing different forms of dextrans such as: dextran with high molecular weight up to 200.000 or low molecular weights dextran up to 40.000, moreover insoluble glucan such as sephadex G-25 up to sephadex G-200 (Kubik *et al.*, 2004, Khalikova *et al.,* 2003, Kolenbrander *et al.,* 2002 and Igarashi *et al.* 2001).

Temperature Dependence of Activity:

The optimum temperature for the activity of crude dextranase on dextran (M. wt 40.000) was found to be 20-23°C Fig. (2) which is one of the lowest temperatures reported for dextranase. Most dextranases showed optimum temperatures from 35 to 75°C (Abdel-Aziz *et al.*, 2007, Erhardt and Jördening, 2007, Hoster *et al.*, 2001, Arnold *et al.,* 1998). This optimum temperature may be applicable in sugar industry as the ambient juice temperature in many factories are 26.7-32.2°C, where most applied dextranases showed low activity at the same temperatures (Eggleston and Monge, 2005). About 40% of the maximal activity of *Streptomyces anulatus* could still be recorded at 4°C, which is temperature that allowing maximal growth of the organism. Cold active enzymes are frequently thermo labile, a fact which is

understandable, since they have to be flexible enough to be active at lower energy cost. In contrast, alkaline tolerant dextranase from *Streptomyces anulatus* isolated from soil of homestead chicken coop, optimum activity was 40-50°C although peaking growth and production was 28-30°C (Andney *et al.,* 2003).

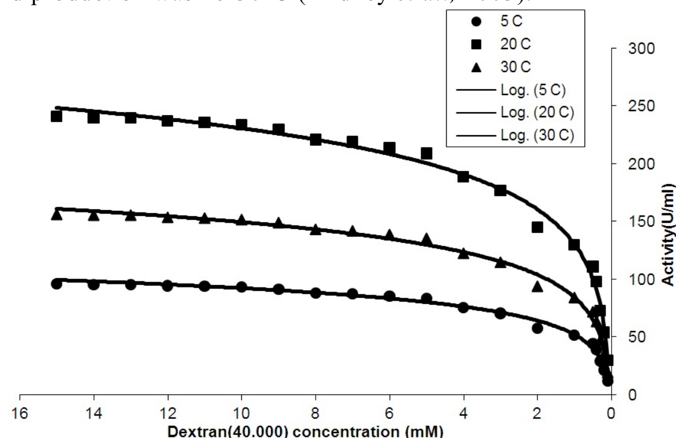

Fig. 3: Effects of different concentrations of dextran on the enzymatic activity at different temperatures.

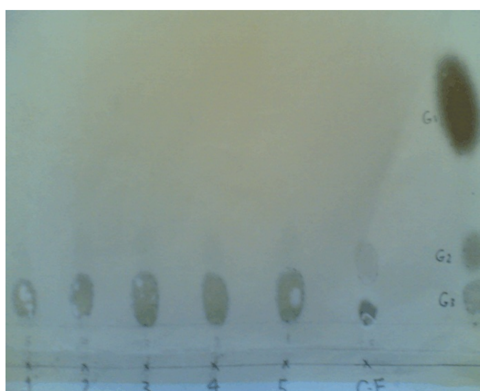

Fig. 4: Hydrolysis product of dextran by the effect of dextranase.
1-Dextran(40.000), 2-Dextran(70.000), 3-Dextran(240.000), 4- DEAE-Sephadex 50, 5- Sephadex G-200, C.F=Culture filtrate after 4 day incubation
G1=glucose, G2=isomaltose, G3=isomaltotriose.

As illustrated in Fig. (3), the enzyme was uninhibited by excess substrate and remained unaffected by changes in temperature. Psychrophilic enzymes counteract the inhibitory effect of low temperatures on activity by reducing the temperature dependence of the reaction rate (Xu *et al.*, 2003). In this respect this direction of research may be of interest in the innovative application of cold-active dextranase in overcoming dextran problems at lower temperature especially in the crystallization process.

Degradation of Dextrans:

Cold active dextranase of *Streptomyces anulatuse* at 20°C was tested against soluble dextrans with average molecular weights 40.000, 170.000, 240.000 and against insoluble dextrans DEAE-sephadex G50 and sephadex G-200. Reducing sugars were released from all of these substrates at the end of incubation time as mentioned before were analyzed by paper chromatography Fig.(4) and TLC, they all were identical. The molecular mass determined that the sugars formed were tetrasaccharide. The Rf values of these saccharide either on paper chromatography or on TLC were identical with that of isomaltotriose i.e. the product of all reaction mixtures was isomaltotriose indicating high performance of the isolated dextranase towards isomaltotriose synthesis.

Accordingly, cold active dextranase of the isolated *Streptomyces anultus* can be classified as extracellular endodextranase (E C 3. 2. 1. 11) because dextranase, 1, 6-α-D-glucan 6-glucanohydrolase, is an enzyme, which catalyzes endo-hydrolysis of α-(1,6)-D-glcoside linkages in random sites of dextran producing isomaltose, isomaltotriose and small amount of D-glucose, together traces of higher oligomers as in mold dextranase or producing only isomaltotriose as in bacteria (Khalikova *et al.,* 2005). Consequently cold active dextranase of the isolated *Streptomyces anulatus* can be used for synthesis of pure and high amount of isomaltotriose. Recently dextranase has gained much interest in the directed synthesis of isomaltotrio-oligosaccharide which have been shown to exhibit prebiotic effects (Goulas *et al.,* 2004, Kubik *et al.,* 2004, Thitaram *et al.,* 2005).

Table 2: The overall performance of the enzyme.

Form of dextranase	Proteinmg/ml	ActivityU/ml	Specificactivity U/mg protein	Amount of isomaltotriose (mg/ml)
Culture filtrate after 1weak incubation	1.175	43.5	37	0.00
Partially purified dextranase(80%NH2SO4)	0.225	230	1022	0.45
Immobilized dextranase (hydroxyapatite)	0.09/mg carrier	113/mg carrier	1255	0.70

Enzymatic Synthesis of Isomaltotriose by Micro Reaction Technology:

Cold-active dextranase was immobilized on powdery hydroxyapatite by the method mentioned before. And packed in a column (1 ml) made from 2ml syringe for testing its performance for isomaltotriose synthesis. The results are summarized in Table (2).The circulation of substrate solution through the immobilized dextranase was performed using syringe pump and resulted in production of 0.7mg isomaltotriose per ml reaction mixture per minute equivalent to specific activity 1255 unit / mg carrier compared with free enzyme which produced 0.45 mg isomaltotriose per ml reaction mixture per minute equivalent to specific activity 1022 unit / ml reaction mixture. This is may be due to that, immobilization of enzyme on a micro space (micro particles of hydroxyapatite) leads to micro channel reaction system took place between enzyme and substrate provide large surface and interface areas which differs from macro-scale systems (free enzyme) with regards to the strict control of reaction time. This rapid mass transfer is the key advantage of micro reaction technology (Miyazaki and Maeda, 2006). In a comparison between repeated batch process (batch wise, in a test tube) and continuous batch process(in a column reactor) for immobilized dextranase, the operational stability was evaluated and the results indicated the durability of the immobilized enzyme in both batches. The retained activity after being used for one week were 73 and 51 % for repeated and continuous batch process respectively. The difference of 22% in activity between both process may be due to that the pressure of substrate solution flow compacted the immobilized particles by time and decreased its efficiency.

The overall, preparation of the immobilized enzyme with powdered material is significantly easier, however, it is unfavorable in large-scale processing because they are susceptible to increasing pressure by increasing flow rate and particles become highly compacted and cause plugging, therefore improvement of the entire process is still required, to obtain the benefit that can be derived from their use and for them to be evaluated as common or standard technology (Schoemaker *et al.,* 2003, Garcia-Junceda *et al.,* 2004). It can be concluded that batch wise dextranase micro-reactor is effective in large scale either for isomaltotriose synthesis or application in sugar factory than continuous micro reactor.

Effect of different sugars on free and immobilized dextranase:

Cane juice is a complex matrix and contains numerous large particles, including starch granules (Eggleston and Harper, 2005). Sugarcane juices have high levels of glucose, fructose, and particularly sucrose. Mannitol will also often be present when dextran is present in cane juice because both are formed mainly from *Leuconostoc mesenteroides* (Eggleston, 2002). These short and long chain carbohydrate sugars could potentially affect the activity of dextranase if applied in sugar factories. Therefore, the effect of different sugars as they approximately occur in sugarcane juices was investigated. The enzymatic activity was assayed as mentioned above in material and methods section in presence of 1% (weight/volume) of different sugars+ 2% dextran (40.000), the results are shown in Table (3).

None of the sugars tested showed any adverse effect on dextranase activity of both free and immobilized enzyme except mannitol and starch. This result confirms the capability of application of cold active dextranase of the isolated strain of Qaron Lake in sugar factories. As indicated in Table (3), mannitol was markedly inhibited the activity of the free dextranase but in contrast, it slightly inhibited the activity of the immobilized dextranase. The relatively large amounts

of mannitol present in deteriorated cane or beet may affect sugar industry processing like dextran or much higher (Bruhns *et al.*, 2004) but this is not the aim of the present work. The aim was focused on overcoming dextran problems with new enzyme and overcoming accompanied problems which might face practical application of the enzyme like presence of high amount of mannitol. Immobilized dextranase solved this problem as it retained 95 % of the activity while free enzyme retained 50 %. Starch also found to had slightly inhibitory effect on free dextranase, this inhibition may be due to competitive inhibition between two substrates (starch and dextran) both are polymer of glucose with different glucosidic linkage. However starch had not inhibitory effect on immobilized enzyme. These results highlight the efficiency and the usefulness of the used immobilization process (new micro reaction technology) and the nature of the carrier used (hydroxyapatite) which protected the enzyme.

Effect of different metals on free and immobilized dextranase:

Free and immobilized dextranase were assayed in the presence of various metal ions at 1 mM concentration. Each enzymatic activity was assayed after the enzyme had been treated with each metal ion at room temperature for 10 min. The values shown are the percentages of the activity without the additives, which is taken as 100 %. The results are shown in Table (4) .Fe^{+2} and Fe^{+3} significantly inhibited the activity of either free or immobilized enzyme. For Pb^{+2}, Cu^{+2}, Al^{+3} ions, free enzyme was inhibited to the extent of 40- 70 %, however immobilized enzyme was protected to the extent of 60- 90 %.These results to some extant similar to the results of maltotriose producing amylase of *Nocardiopsis Sp*.(Mitsuiki *et al.*, 2005).

In comparison with dextranase of *Flavobacterium* (Kobayashi *et al.*, 1983), aluminum chloride had slightly inhibitory effect with 97% residual activity, however retained 70 % residual activity of the isolated dextranase. Moreover with cobalt retained 88% for the former and 75% for the later. In contrast, Cupper chloride had completely inhibited the dextranase activity of *Flavobacterium* and inhibited half of the activity of the dextranase of the isolated strain. Only Mn^{+2} and Mg^{+2} had stimulatory effect on dextranase as they retained 120 and 144 % of the original activity respectively, this result is coincide with the results that reported by Kobayashi *et al.* (1983) for dextranase of *Flavobacterium* .

Table 3: Effect of different sugars on free and immobilized dextranase.

Sugars	Free dextranase (Relative activity, %)	Immobilized dextranase (Relative activity,%)
Sucrose	100	100
Glucose	100	100
Fructose	100	100
Sucrose+ glucose+ fructose	100	100
Mannitol	50	95
Starch	83	100

Table 4: Effect of different metals on free and immobilized dextranase.

Metal	Free dextranase (Residual activity %)	Immobilized dextranase (Residual activity %)
Control	100	100
$FeCl_2$	4	19
$FeCl_3$	8	23
$CoCl_3$	75	82
$MnCl_2$	120	118
$PbCl_2$	40	60
$CuCl_2$	50	78
$AlCl_3$	70	90
$MgCl_2$	144	140
$HgCl_2$	33	40

Impact of Cold-Active Dextranase of Qaron Lake Isolate:

Some steps during sugar processing in most factories require low temperature and these steps are the week points where dextran can formed. Our idea proposes that the application of cold active dextranase on factory scale in these stages is effective because the cold active enzymes are adapted to work actively at lower temperature and hence any dextran forms in this temperature will auto-hydrolyzed by the enzyme. This proposed approach will be the first triumphs in overcoming dextran formation in sugar processing especially that this cold active dextranase is energy saving and succeeded on laboratory scale. Cold active dextranase may improve sugar industry and its processing by increasing sugar recovery and sugar purity which will be reflected on the price of produced sugar and hence the economy. Moreover the high performance of either free or immobilized form of cold active dextranase for isomaltotriose synthesis.

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