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THE INVERTASE- A REVIEW

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ABSTRACT

Invertase is an enzymes that catalyzes conversion of sucrose into glucose and fructose, is glycoprotein that works on pH 4.5 and stability at 50ºc. it is broadly delivered in the biosphere in mostly microorganisms and plants. The present studies centered upon invertase together with source of production, isozymes, kinetics, media optimization, types of fermentation, purification methods and applications.

Key words: Bacterial Invertase, Beakers Yeast, Chromatography, Purification.

INTRODUCTION

All living cells enclose convoluted globular protein named as enzymes, functions as biocatalyst expedites biochemical reactions in vivo and in vitro. Kuhan titled such biocatalyst as enzyme from Greek word, “enzumas” which point out brewing of bread by yeasts. The expedite nature of enzymes are accountable for the working. It cooperate in reaction without being depleted in the reaction, getting very high speed of product formation by decreasing the Gibbe’s free energy (Δ Gº) required to start the reaction.[1] Enzymes are very specific in nature due to this it can discriminate between chemicals with very much alike structure and expedite the reaction over different temperature range (0-110 **ºC**) and in wide pH ranges (2-14). In industries, such properties with an enzyme being harmless and bio-degradable can results in large quantity and excellent stuff. Also an enzyme can be achieved from various microorganisms and that also enormous amount without applying any chemical resistant methods **[1].**

The industrial knowledge of enzymes rotates around malt and yeast where classical baking and leavening industries were immediately expanding. Much of the early progression of biochemistry was focused on yeast fermentations and mechanism for conversion of starch to sugar or cellulose to sugar **[2].** one such types of enzyme of our attraction is “Invertase”. This review centered on the catalytic nature, extraction methods and its application in today’s globe.

The basic source of all living organism is carbohydrates. Monosaccharide like glucose and fructose are primarily used in metabolism **[3].** Thus, invertase plays important role as it is sucrose degrading enzymes. β-fructofuranosidases (EC.3.2.1.26) or Invertase are special type of enzymes that drive the decompounds of sucrose into commixture of sucrose and glucose titled as inverted sugar **[4].** The decompounding of b-D-fructuranosides (stachyose and raffinose) also accomplished by invertase whose products are beneficial in creation of bakery stuff and helps in brewing of sugarcane molasses **[5].** Enzymatic action of invertase has been outlined chiefly in microorganism and plants.

Sources:

Plant Invertase:

There are three types of invertase namely, vacuolar, cytoplasmic and cell-wall, have been extracted from various species and characterized as biochemical level. Acid invertases have been extracted from various plants species. Soluble acidic invertase has been purified from many plant species, All soluble invertase inspected have a Km for sucrose in the mill molar range at a pH optimum between 4.5 and 5.5. In addition, all soluble invertase seem to be N- glycosylated proteins.

Table 1: Different plants sources were used to isolates invertase.

Plant species	Km in (mM)	References
Papaya	7.7	**[6]**
Sugar cane	2.8	**[7]**
Oat	4.58	**[8]**
Maize	1.84	**[9]**
Potato	16	**[10]**

Bacterial sources:

There are various bacteria present in soil like Bacillus, and Bacillus cereus for invertase production. It is also found there are some gram negative bacteria those can discharge invertase enzyme relates to the order of actinomycetales such as Arthobacter species, Barevibacterium SP.

Table 2: Following bacteria were used for production of invertase enzyme.

Bacteria	Country &year	References
Arthrobacter globiformis IFO 3062	2004 Japan	**[11]**
Arthrobacter sp. 10137	China 2009	**[12]**
Bacillus cereus TA-11	China 2007	**[13]**
Bacillus macerans	2004 Cairo, Egypt.	**[14]**
Bifidobacterium infantis ATCC 15697	, Ireland 2005	**[15]**
Brevibacterium divaricatum NRRL B	ND	**[16]**
Lactobacillus brevis Mm-6	ND	**[17]**
Thermotoga neapolitana	ND	**[18]**

Yeast sources:

Yeast is good sources of invertase enzyme production. Particularly Yeast has become very common source for invertase enzyme. Among the researchers many are utilizing yeast as rich source.

Table 3: Following yeast are used to produce invertase enzyme.

Yeast	Country year	References
Candida guilliermondii	México. - 2014	**[19]**
Hansenula polymorpha	2003 china	**[20]**
Kluyveromyces marxianus	Pakistan 2010	**[21]**
Leucosporidium antarcticum	2005 Honolulu	**[22]**

Kinetics of Invertase:

Invertase show high catalytic activity over a wide range of pH (3.5-4.5) with the best pH of 4.5. The enzyme activity attains an optimum at 55 ºC. The Michaelise Menten (Km) value for enzyme is 30 mM (approx.). This enzyme is glycoprotein stability shows at 50 ºC. some important cataions like Hg^{2+} Ag^{+},Ca^{2+} and Cu^{2+} works as enzyme inhibitors **[23].** The fructose analogue 2, 5-anhydro-D-mannitol shows Competitive inhibition instructing that the enzyme activity was forbidden by the furanose. $AgNO_3$ and $HgCl_2$ scrutinized as a total inhibiter of the enzyme while $CuSO_4$ and $Cd(NO)_3$ exerted a 76% and 73% inhibition, respectively **[23].**

Table 4: Some invertase inhibitors are listed here.

Bacterial strain	**Inhibitors**	**References**
Arthrobacter globiformis IFO 3062	ND	**[11]**
Brevibacterium divaricatum NRRL B-2312	$Cu^{2+,}$ SH	**[16]**
Bacillus cereusTA-11	Hg^{2+}, Cd^{2+} , Cu^{2+}	**[25]**
Bifidobacterium breve UCC2003	ND	**[24]**
Bifidobacterium infantis ATCC 15697	Cu2+, $Hg2^{+}$ pCMB	**[26]**
Lactobacillus reuteri CRL 1100	$Ca^{2+,}Cu2^{+,}Cd^{2+,}$ $Hg^{2+,}$ β-ME, DTT	**[27]**
Thermotoga neapolitana DSM 4359T	2 mM$CuSO_4$, $ZnSO_4$, $FeCl_2$, HgCl2,Urea,	**[28]**

Influence of pH on bacterial invertase activity:

Many research papers outlined the best pH for invertase enzyme activity is neutral pH with some exceptions .there were wide range of pH buffer applied to scrutinized the invertase activity. Different bacterial invertase works well on specific PH.

Table 5: Effects of pH on invertase enzyme

Microorganism	**pH and temperature**	**Km**	**References**
Brevibacterium divaricatum NRRL 2312	pH 6.8; 40 °C, more than 95% of the initial activity was at 30 °C and less than 15%at 50 °C	0.19M	**[16]**
Arthrobacter globiformis IFO 3062	pH 6.8; 37 °C	Km 2.4Mm ,k0 127 s	**[11]**
Bacillus cereus TA-11	pH 7.0; 50 °C	370 mM, 3.0 µmol	**[25]**
		min−1	
Bifidobacterium breve UCC2003	pH 6.0; 37 °C	Km-25mM 24 µmol/min/mg	**[24]**
Bifidobacterium infantis ATCC 15697	pI 4.3; pH 6.0; 37 °C	Vm/Km = 0.65 and 0.025 × 10−3 min−1 mg−1	**[26]**

Isozymes:

In yeast, there are two form of invertase that is extracellular invertase and intracellular Invertase **[29].** Extracellular invertase is glycoprotein containing about 5% mannose, 50% carbohydrates, 3% glucosamine whereas intracellular invertase contains no carbohydrate. it has been scrutinized that in depressed yeasts invertase is extracellular and in well repressed state all invertase are intracellular. Bothe intracellular and extracellular enzymes are inhibited by iodine and mercaptoethanol reactivates it **[30].**

In plats, many isoforms of invertase enzyme stands with different biochemical idiosyncrasy. On the premise of solubility, isoelectric points, best pH and sub cellular location, plant invertase can be categorized into three subgroups. These subgroups are- acid soluble (vacuolar), alkaline soluble (cytoplasmic) and cell wall bound Invertase **[31].**

Invertase enzyme production from bacteria:

Optimized media for bacterial sources:

There is very less written matter focusing on media optimization of leavening condition for bacterial invertase production. Some studies have proclaimed Bacillus species an excellent source for invertase production and approved by food and drug administration. Bacteria are very fastidious in nature owe to this huge production of invertase enzyme can be enhanced by optimizing best production media. There are some production media has been used by researchers to enhance the production of invertase enzyme.

Table 6: Isolated bacterial strain, kind of enzyme, source and optimized production media

Bacteria	Kinds of enzyme	Bacteria l source	Culture mediu m	Refer ences
Arthrob acter globifor mis IFO 3062	Extrac ellular		0.2%ye ast extract, 1% polype ptone, 0.4%($NH_4)_2HPO_4$, 0.1% $MgS0_4$ $7H_2O$,	**[11]**
Arthrob acter sp. 10137	Extrac ellular	Not identifie d	4% corn steep powde r, 0.13% $MgSO_4$. $7H_2O$, yeast extract, 0.4%($NH4)_2HPO_4$,4%s ucrose	**[12]**

Bacillus cereus TA-11	Intracellular	Soil	0.6% yeast extract, 0.1% $KH2PO_4$,0.1% K_2HPO_4 SY broth with 1% sucrose	**[25]**
Bacillus macerans	Intracellular	National Research Centre (NRC) Cairo, Egypt	3%sucrose,0.5% peptone, 0.3% yeast extract	**[14]**
Bifidobacterimbreve UCC2003	Intracellular	Infant nursling stool	0.05% L-cysteine-HCl, Modified Rogosa-Sharpe medium with 1% sucrose,	
Bifidobacterim infantis ATCC 15697	Intracellular	American Type Culture Collection (ATCC)	Synthetic media with 2% fructose	**[26]**
Brevibacterim divaricatum NRRL B-2312	Intracellular	Northern Regional Research Lab (NRRL)	1% urea, , 0.01% biotin, 0.3% KH_2PO_4 7% sucrose,	**[16]**
Lactobacillus brevis Mm-6	Extracellular	Milk of breast	10% sucrose, 0.2% yeast extract	**[17]**
Lactobacillus reuteri CRL 1100	Intracellular	CERELA	MRS broth added with 1% sucrose	**[32]**

Streptomyces sp. ALKC 8	Extracellular	Sugarcane field soil	Czapek-Dox agar (CDA)	**[33]**
Thermotoga neapolitana DSM 4359T	Intracellular	Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany)	Resazurin 0.0001%, , 0.02% $MgCl_2$.$6H_2O$,0.1%NH_4Cl, 0.03% K_2HPO_4,0.03% KH_2PO_4, 0.01%$CaCl_2$.$2H_2O$,0.1%cysteine-HCl, 0.2% yeast extract, 0.01% KCl,0.2%tryptone,0.5%glucose,vitamin,1% NaCl	**[33]**
Zymomonas mobilis CDBB-B 603	Extracellular	CINVESTAV-IPN, Mexico	0.16%$(NH_4)2SO_4$, 5% glucose, 0.1% $MgSO_4 \cdot 7H_2O$, 0.25 KH_2PO_4, 0.7% bacto-yeast extract	**[33]**

Favorable factors for fermentative condition:

Bacterial invertase production can be enhanced through the fermentation by optimizing the best factors those affects the fermentation process.

As compiled in the table, the production of bacterial invertase is positively affected by best incubation period (12-72h), optimum pH (4.5-9.7) and wide range of temperature (25-50).

Affects of agitation rate and inoculation level were not mentioned in most of the literature, however 120 -250 rpm helps the growth of aerobic bacteria.

Table 7: Optimized fermentation conditions for invertase production

Microorganism	incubation	T°C	pH	Agitation rate rpm	Inoculum size	C source	N source	Reference
Zymomonas mobilis CDBB-B 603	ND	30	4.9	No agitation	ND	5%glucose	7%bacto-yeast extract,0.16% $(NH_4)_2SO_4$	**[11]**
Streptomyces sp. ALKC 8	24	37	5.0	ND	3 discs (9 mm)	1% sucrose	$NaNO_3$ + yeast extract	**[32]**
Arthrobacter globiformis IFO 3062	36	25	7.0	200	ND	ND	1%polypeptone,0.2%yeast extract,0.4% $(NH_4)_2HPO_4$	**[11]**
Bacillus cereus TA-11	36	50	9.5	100	ND	1% sucrose	0.6%yeast extract	**[25]**
Bifidobacterium infantis	16	37	6.8	ND	5%	2%fructose	Semi synthetic medium	**[26]**
Lactobacillus brevis Mm-6	72	30	8.0	ND	2% (1.2 × 106 CFU ml−1)	10% sucrose	2% malt extract, 2% peptone	**[17]**
Arthrobacter sp. 10137	20	30	ND	250 (further increased by 20%)	5%	4% sucrose	4% corn steep powder, 0.4% $(NH4)_2HPO_4$	**[12]**

Influence of agitation rates on invertase production by p.expansum after 48 hr in culture media containing molasses as carbon source, yeast extract as nitrogen source, at 35ºc, initial pH 5 a was inspected by using various agitation rate at 50,100, 250, and 300 rv/min. Maximum invertase production was found at 150 rv/min.

Enzyme purification:

Enzyme purification is process in which contaminates are removed to inspect the nature, function and structure of enzyme. Enzyme activity is enhanced by good purification strategies.

Table 8: Enzyme purification approaches for bacterial invertase

Purification methods	Bacterial strain	Purification	Yield %	Specific activity	Reference
SephacrylS-100; HPLC,Q-Sepharose F.F	*Thermotoga neapolitana DSM 4359T*	469.5	25.8	51,833.5	**[28]**
Gel filtration (Sephadex G-75), Ammonium sulfate precipitation (40–80%); DEAE chromatography (A-50);	*Bacillus cereus TA-11*	15.37	26.6	207.5	**[25]**
Gel filtration (Sephadex G-);Ionexchange chromatography (DEAE-Sephacel), Ammonium sulfate precipitation (80%);	*Lactobacillus reuteri CRL 1100*	31.2	16.9	9.2	**[27]**
Gel filtration (Sephadex G-I00),Chromatography on DEAE-Sepharose CL-6B, Toyopearl HW-65F Bio-Gel P-I00;	*Bifidobacterium adolescentis G1*	202	20.2	101	**[34]**

DEAE-cellulose chromatography; Ammonium sulfate precipitation (48–70%);	*Brevibacterium divaricatum NRRL B-2312*	345	8.2	345	**[16]**

Production of invertase by submerged fermentation:

Currently, various microorganisms are desirable for production of invertase rather than plants or animals because of less toxic compounds are produced during fermentation process **[35].**

Now days mostly submerged state fermentation process are used in invertase production compared to solid state fermentation **[36].**

On huge scale, submerged state fermentation is a classical fermentation process for production of invertase. It is profitable technology with maximum yielding per reactor volume and can be freely inflict to culture handling and downstream processing. In this fermentation production, the tabbed microorganism is cultured in sealed vessels that encompass oxygen and nutrient broth **[37].** Invertase producing microorganisms utilizes nutrients for production of invertase and discharges it into the fermentation medium.

Differently, solid state fermentation manages microorganisms on solid base instead to liquid medium. This strategy arranges an environment that is very much alike to microbe's natural environment and appropriate for the growth of microorganism that need less moisture. Solid state fermentation is not adopted in immense production of invertase due to its restrains including deadlock to standardize the operations. The rising attentions on pollution and to examine cost effect have expanded the curiosity on application of waste materials for the production of Invertase **[38].**

Various waste materials and fermentative approaches have been out lined for invertase production as shown in table-

Table 9: Different waste materials and fermentation methods.

Submerged fermentation

Substrate	Microorganism	Maximum production condition	Production	References
Agro-industrial wastes	*A. niger*	pH6.5 at 25°C for six days	15.9±2.44 u/g	**[39]**
Fruits peel	*A. niger*	pH5.0 at 30ºC for four days	16.25±0.60 µM	**[40]**
Molasses	*Fusarium solani*	pH5.0 at 30°C for four days	9.90 U/mL	**[41]**
Agriculture-based by-products	*A. flavus*	pH6.5 at 40°C for two days	7.41 U/mL	**[42]**

Solid state fermentation

Substrate	microorganism	Production condition	yield	References
peels of fruit	*A. niger*	Four days at 30c ,pH 5	51 U/mL	**[43]**
Residue of red carrot	*S. cerevisiae*	72 hr at 30c	272.5 U/g	**[43]**
Residue of sugercane	*S. cerevisiae*	Three days at 40c ph 5	430 U/mg	**[36]**
Wheat bran	*A. niger*	Three days at 30c ph 5	194.71 U/g	**[45]**

It is difficult to find out the kinds of waste materials or strategies of fermentation compatible for invertase production since these researches are conducted in pilot scale using bioreactors. Thus the application of much alike approach in coming days will give more important information

Application of invertase:

Now day's enzymes are used in industrial actions because of their gentle performing condition like pH and temperature. In industries, Invertase has been employed in good application like food beverages, pharmaceutical and biosensor.

In beverages:

In beverages industries, invertase has been broadly used in manufacturing of fructose or invert syrup. Invertase is employed as and preferably to the classical acid hydrolysis approaches which might discharge unwanted by- products. Because of hygroscopic nature of invert sugar it is widely used in production of soft candies, jams, chocolates **[36].**

Pharmaceutical industry:

Differently, invertases are crucial enzyme in pharmaceutical industries for drug formulation. On the other hand, importance of invertase in pharmaceutical is enhanced by analysis of invertase with transfructosylating activities which generate FOS. FOS is an oligosaccharides made up of tiny fructose chains that is less sweeter than sucrose, less in calories, preparing them excellent for diabetic patients **[46].**

Biosensor:

Invertase can help as biosensor for identification of sucrose in a simple and fast way rather than traditional methods such as electrophoresis and chromatography.

Bagal et al. (2015) stated the fiction of nano – gold cluster mediated by invertase, incorporate on onion membranes. This reported hypothesis can be employed as sucrose biosensors based on fluorescence **[47].** Appropriate to more than one cancer is not an impossible achievement.

CONCLUSION

Invertase enzyme available in wide range of organisms encompasses immense commercial importance, especially in pharmaceutical and food industries. Different studies have earlier determined a full description of this enzyme but modern investigations which centered on sources of invertase that helps in isolation of invertase from different sources and might have exclusive trait such as transfructosylating action that will enhance the commercial value of this enzyme.

Furthermore, studies on isozymes of invertase and kinetics of invertase might be useful in creation artificial enzymes in future. Through the media optimization and type of fermentations and purification processes, yielding of invertase production can be enhanced by following compatible parameter.

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