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COMPARATIVE STUDY OF DIFFERENT CARBON SOURCES ON PRODUCTION OF BETA GALACTOSIDASE FROM FUNGAL ISOLATES.

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Abstract:

Beta galactosidase is one of the most abundant enzymes present in nature. It is found in many organisms right from bacteria, fungi, bugs, plants, and animals. Fungi have various industrial usages as source of enzyme, protein, antibiotics and even bioremediation clearing pollutants. It is responsible for breaking down of disaccharide lactose to produce galactose and glucose. These end products then enter the glycolysis cycle. These enzymes are located into the lysosomes compartments into the cell where recycling of different types of molecules take place. In this study the comparative analysis of impact of different carbon sources on the production of beta galactosidase from fungal isolates was performed. Although the prime aim is to develop complete system for enzymatic degradation of cellulosic wastes from the paddy and wheat straw, however, the system needs initiation for the microbial growth and production of the beta galactosidase enzyme. Initial mixing of the simpler carbon source can help enhance the overall production of the desired enzyme. It is important for the commercial production of the beta galactisidase enzyme. The fungal strains were isolated from the soil samples and were screened for the production of beta galactosidase enzyme at the extracellular levels. The top 3 isolates were then checked for their stability into production of the enzyme at larger quantity. Also, the fungal isolated PT1, PT2 and PT3 were checked for greater enzyme production and stability at higher temperature. This was important experiment at most the industrial wastes are at higher temperature and need better utilization ability in the strain to be used at that temperature. To determine which carbon source has maximum positive impact on the overall production of the beta galactosidase enzyme by the top producer isolates, choice of glucose, lactose and sucrose was taken. The PT2 isolate showed the best results as expected from among all three isolates. But the difference was observed among the three as PT1 and PT3 showed little rise into the production of the beta galactosidase enzyme on shifting from glucose to sucrose to lactose as source of carbon. The PT1 strain showed comparatively higher jump into the production of the enzyme when compared with those two strains. Also, the difference into the jump was when the concentration was increased to 5ml/ml from 2mg/ml of lactose into the media. This could be probably because the PT2 contains gene for beta galactosidase production, which gets induced under the higher concentration of lactose than any other isolates. This could also mean that lactose can be mixed with the paddy straw and wheat straw substrates to boost the production of the beta galactosidase enzyme. It can also help speed

up the process of naturally degrading these agrowaste materials that are burned by the farmers to produce pollution.

Introduction:

β-Galactosidase (β-D-galactoside galactohydrolases, EC 3.2.1.23) is an enzyme that catalyzes the cleavage of terminal galactosyl groups from the non-reducing ends of different galactosides (**Ansari and Satar, 2012)**. The enzyme is ubiquitous in nature, and can be derived from various sources such as plants, animal organs and microorganisms.

β-Galactosidase in plants

Wehmer and Hadders have recorded the gatherings of the plant kingdom wherein the protein is known to occur. The limit of β-Galactosidase in plants is acknowledged to be to hydrolyze glycosides which have the beta-linkage. It furthermore catalyzes association (transferase) reactions in plants.

β-Galactosidase in animals

The fundamental site of β-Galactosidase activity in animals is the stomach related tract where it hydrolyzes lactose from the eating routine. The impetus in like manner is accessible in the pancreas, kidney, adrenal, thyroid, spleen, liver, testis, epididymis, vas deferens, and male frivolity releases.

Cohen et al. declared that enzymatic development, when present, was in the cytoplasm of epithelial "cells and was missing from centers, connective tissue, and muscle. β-Galactosidase development is much higher in the stomach related arrangement of the fetus and suckling animal. This closeness of high activity matches with the time that milk outlines the major or entire enhancement hotspot for the animal.

β-Galactosidase in humans

β-Galactosidase is accessible in adult human salivation and stomach related organs. A couple of individuals have low β-Galactosidase development in their absorption tracts which results in lactose bias. The rate of low β-Galactosidase development among individuals is significantly higher in Negroes, American Indians, Greek Cyriots, and Asians.

B-Galactosidase in microorganisms

Because of the wide work with β-Galactosidase, it won't be possible or feasible to allude to all examinations drove or even the microorganisms investigated. Therefore, only a segment of the more applicable and progressing examinations on a segment of the more regular microorganisms of eagerness for the dairy business will be considered. Characteristics of the β-Galactosidase systems of these microorganisms will be differentiated and results gained in this examination on

β-Galactosidase of *P. shermanii.* Examinations on the going with microorganisms are very convincing: E. coli.

β-galactosidases are extensively being used in food and pharmaceutical industries due to their capability to hydrolyse lactose, the most abundant sugar in milk and its by-products. This enables alleviation of lactose intolerance problem, prevalent in high share of the human population, and thus broadens consumption of these products (**P.S. Panesar., et al. 2006 and 2010**).
This enzyme is also used in toxicity tests and pharmaceutical industries, making it one of the commercially important enzymes in the world. It also helps solve many health related issues (**Harper 2nd Ed. 1981, Apartin et al., 2001 and Hatzinikolaou et al., 2005**).
The β-galactosidase utilization also includes the treatments of wastewaters from dairy industries. Namely, disposal of whey and whey permeates causes huge problems in the environment, owing to the low lactose biodegradability. Hence, hydrolyzed whey lactose provides solution for the problem of pollution (**Q. Husain. et. al., 2010 and M.R. Kosseva et al., 2009**).
Every organism derives its nutrition from the food it eats. Higher animals consume plants and lower organisms as source of nutrition and energy. Microorganisms utilize organic and inorganic sources for their energy and nutrition. Carbon is the building block of the nature and almost everything is made up of carbon and it's by products. The cells take up the carbon from different sources as substrates for their metabolic activities. Here they are broken down into supply pool of amino acids and other important components for the cell. Amino acids take up a majority of carbon supply (about 55%) (**Neidhardt et al., 1990**). The enzymes are produced as secondary products in the microbes that are useful for the humans in various forms. The β-galactosidase is one such enzyme with many uses for the humans. And since its protein in nature most of amino acids help into protein formation in the cells. Enzymes also need amino acids for their formation. Carbon sources that can be readily absorbed and used by the cells are most preferred. For our commercial purpose the ones that help increase the yield of the desired product is the preference. In this case, the carbon source that helps increase the production of beta galactosidase enzyme. The optimization of the medium for the highest production of beta galactosidase enzyme is very important for industrial level production. It should also have higher stability and functionality over a wide range of substrate mixture and concentrations.

Review Of Literature:

In the metabolic network different carbon sources enter at the different metabolic levels to perform their true functionality (**Neidhardt et al., 1990, Schonheit et al., 2016 and Lehninger, 2008**).
To study the effect of different sources of carbon on the production of lactase, glucose, galactose, sucrose, maltose, fructose, and xylan were separately added to the medium. Although the growth rates were within close proximity, the lactase-specific activity was found to be higher in the medium containing lactose as compared to those of other sources of carbon. This study was done in T. viride (**Isıl Seyis et al., 2007**). The study also compared different nitrogen sources in the medium having impact on the production and activity of beta galactosidase enzyme.

In a study on effects of carbon source on expression of f_o genes and on the stoichiometry of the c subunit in the F_1F_o ATPase of Escherichia coli. Translation rates of both uncB and uncE change as culture density increases, but transcription rates do not. Quantitation of the c stoichiometry shows that more c subunits are assembled into the F_1F_o ATPase in cells grown on glucose than in cells grown on succinate. E. coli therefore appears to have a mechanism for regulating the composition and, presumably, the function of the ATPase in response to metabolic circumstances (**Schmidt et al., 1998**).

A fungal strain isolated from rotten banana and identified as Aspergillus alliaceus was found capable of producing thermostable extracellular β-galactosidase enzyme. For immobilized enzyme-substrate reaction, these three variable, temperature, time, and pH were optimized at 50 °C, 40 min, and 7.2, respectively. Glucose was found to inhibit the enzyme activity. The K(m) values of partially purified and immobilized enzymes were 170 and 210 mM, respectively (**S. Sen et., al., 2012**).

In a study conducted with ***A. nidulans***, the lactase activity was found to be higher in lactose containing medium. In this study, it was also indicated that galactose has a positive effect on enzyme activity (**Fantes et al., 1973**).

In a study carried out with ***Auerobasidium pullulans***, it was concluded that lactose is a stronger inducer for enzymes than are other sugars. It also helped to understand the focus points into optimization of sugar sources into the media for the production of beta galactosidase enzyme (**Deshpande et al., 1989**).

Since bacteria are also a rich source of beta galactosidase enzyme, a study by **Mukesh Kumar et. al., 2012**, found that xylose was the best among the different carbon sources they took. This was followed by maltose as the carbon source. The microbe used by them for this study was ***Bacillus spp.*** that they isolated from dairy plant soil.

In a study on various nutrients belonging to three categories, carbon, nitrogen and amino acid sources, were investigated in terms of their effect on the production of extracellular β galactosidase by ***Bacillus licheniformis*** **ATCC 12759**. Amongst simple carbon sources, xylose and galactose supported maximum β-galactosidase production. Comparison with the control there was significant increase in enzyme yield in the case of the supplementation complex carbon source such as rice flour (**Nurullah AKCAN, 2011**).

Fungal culture exhibiting β-galactosidase activity was isolated and identified as **Aspergillus oryzae** from soil polluted with milk dairy factory effluents. Optimization of β-galactosidase (E.C 3.2.1.23) was carried out in Solid State Fermentation (SSF). Wheat bran and rice husk supports the maximal growth and β-galactosidase production by ***A.oryzae***. The fungal culture utilized several carbon sources for the β-galactosidase induction. Glucose serves as a best carbon source, followed by lactose, maltose and sucrose (**Nizamuddin et al., 2008**).

In a study where the objective was for the production, purification, and characterization of an extracellular β-galactosidase from a filamentous fungus, *Aspergillus niger*. The enzyme production was optimized by a factorial design. Maximal β-galactosidase activity (24.64 U/mL) was found in the system containing 2% of a soybean residue (w/v) at initial pH 7.0, 28 °C, 120 rpm

in 7 days. The results revealed a ***A. niger*** β-galactosidase obtained from residue with favorable characteristics for food industries (**Martarello et. al., 2019**).

In a finding in **1981 by Basil,** the ***Fusarium moniliforme*** produced extra cellular lactase enzyme. They cultivated it on the whey liquid medium or on wheat bran solid medium with the optimum pH between 4-5. He also found that 30^{o}C was the optimum temperature for the production of beta galactosidase enzyme.

Various nutrients belonging to three categories, carbon, organic nitrogen and complex organic sources, were investigated for the first time in terms of their effect on the co-production of extracellular thermostable alpha-amylase and beta-galactosidase by ***Bacillus subtilis***, a bacterium isolated from fresh sheep's milk. The work was furthered for selection of appropriate carbon and nitrogen sources or other nutrients is one of the most critical stages in the development of an efficient and economic process (**Konsoula et al., 2007**).

Material and Method:

The fungi were isolated from the soil samples taken from agricultural fields in Maharashtra. The fungal isolates were tested for their ability to produce extracellular beta galactosidase enzyme. Top 3 fungal isolates with maximum production were further taken for study of the impact of different carbon sources on production of beta galactosidase in them. To determine the production level crude enzyme extracts were prepared in equal quantity and the overall degradation of the substrate is measured to determine the quantity and activity of the enzyme produced by each fungal isolate.

Isolation of Fungal Strain From Soil:

i. The fungi were isolated using the soil dilution plate method by Waksman, 1992.
ii. After testing for the production of beta galactosidase enzyme by the fungal isolated they were sub-cultured on Potato Dextrose Agar Slants. iii. Each pure culture was then preserved at 4 °C as glycerol stock.

Effect of carbon sources on the production of β-galactosidase:

Initially Nutrient Broth media 500 ml was prepared pH was maintained at 6.8 and 250 ml conical flasks were washed and autoclaved along with the media. Then 100ml of media was poured in the respective flasks and the carbon sources sucrose, glucose and lactose were weighed for 2 different concentrations 2mg/ml and 5 mg/ml. then sucrose, glucose and lactose were added in the media and mixed properly. The conical flasks were again autoclaved. After that the isolates PT1, PT2 and PT3 were inoculated in the flasks and kept for 72 hours incubation at 28^{0}C. After incubation, the readings were taken by using spectrophotometer and the production was calculated.

Testing For The Betagalactosidase Production:

i. X-gal (5-bromo-4-chloro-3-indoxyl-D-galactopyranoside) is a substrate, which has been used to screen β-galactosidase positive organisms.
ii. 50 µl of X-gal (20mg in N-N dimethyl form amide) solution was poured over the Nutrient agar.

iii. This medium was incubated at 37 ^{0}C for one hour.
iv. 50 µl from each isolate was poured over Nutrient Agar. v. Media were incubated for 72 hrs for color formation. X-gal forms blue color if the culture has β-galactosidase activity.

Enzyme Assay:
Crude Enzyme Extraction:

i. The enzyme was extracted by mixing by 1 kg of fermented matter with distilled water (1:10 volume) at approximate room temperature and agitated for 1 h on a rotary shaker (180 rpm).

ii. The mixture was filtered through a double-layered muslin cloth and cotton wool and it was centrifuged at 10,000 rpm for 10 min.
iii. The clear supernatant obtained was used as the crude enzyme.

β-galactosidase Activity Assay:
i. 50 µl of 10 mM PNPG in 50mM Sodium Acetate buffer at pH 4.
ii. 50 µl of enzyme extract was added
iii. The mixture was incubated for 30 minutes at 37 o C.
iv. The reaction was stopped by adding 500 µl of 1M sodium carbonate
v. The absorbance was measured at 420nm
Formula for calculating β-galactosidase Activity:
One unit of Enzyme = 1000*OD420nm/t*V
Where t = time of incubation
V = volume of the assay sample

Result:
For Lactose

Sample	**Abs 420nm Set 1**	**Abs 420nm Set 2**	**Abs 420nm Set 3**	**Carbon source**	**Concentration (mg/ml)**	**Activity**
Blank	0.002	0.003	0.001		2	
Isolate PT1	0.850	0.852	0.852		2	47.29
Isolate PT2	1.122	1.125	1.122	**LACTOSE**	2	62.38
Isolate PT3	0.953	0.953	0.953		2	52.94
Sample	**Abs 420nm**	**Abs 420nm**	**Abs 420nm**	**Carbon source**	**Concentration (mg/ml)**	**Activity**

Blank	0.002	0.001	0.001		5	
Isolate PT1	0.860	0.860	0.860		5	47.77
Isolate PT2	1.302	1.302	1.302	**LACTOSE**	5	72.33
Isolate PT3	0.957	0.957	0.956		5	53.14

Figure.1.

For Sucrose

Sample	**Abs 420nm**	**Abs 550nm**	**Abs 600nm**	**Carbon Source**	**Concentration (mg/ml)**	**Activity**
Blank	0.002	0.003	0.001		2	
Isolate PT1	0.847	0.847	0.847		2	47.05
Isolate PT2	1.117	1.116	1.117	**Sucrose**	2	62.03
Isolate PT3	0.950	0.950	0.950		2	52.77
Sample	**Abs 420nm**	**Abs 420nm**	**Abs 420nm**	**Carbon Source**	**Concentration Mg/ml**	**Activity**
Blank	0.002	0.003	0.001		5	
Isolate PT1	0.850	0.850	0.850		5	47.22
Isolate PT2	1.200	1.200	1.201	**Sucrose**	5	66.66
Isolate PT3	0.954	0.954	0.954		5	53

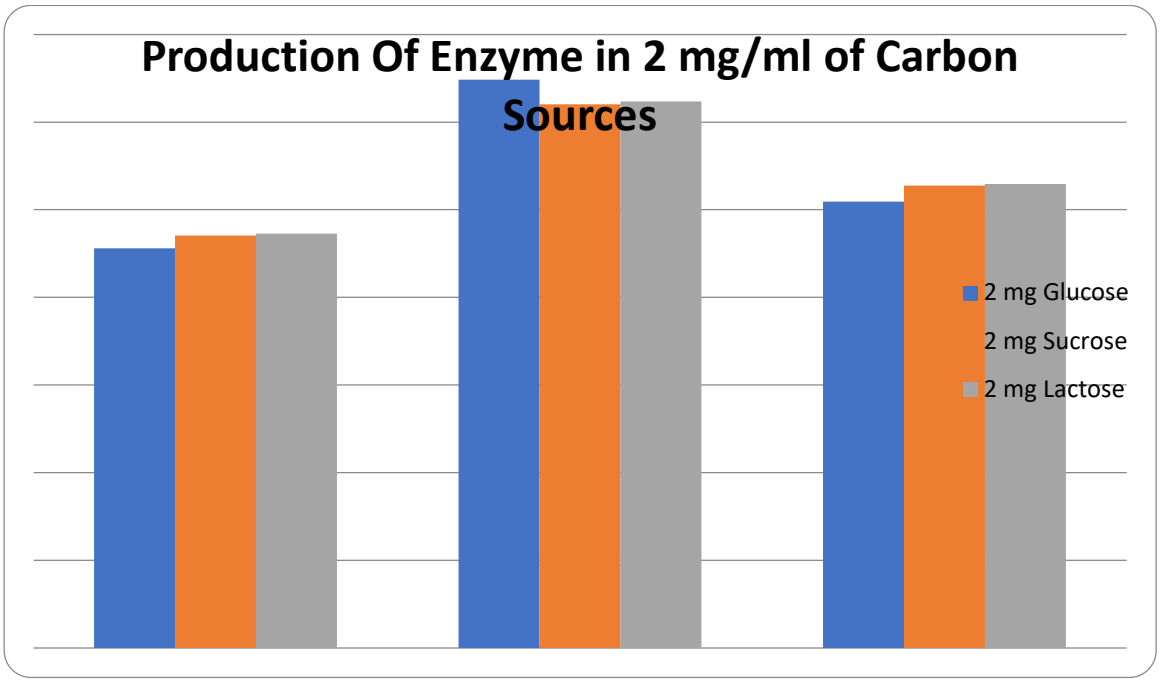
Figure.2.

For Glucose

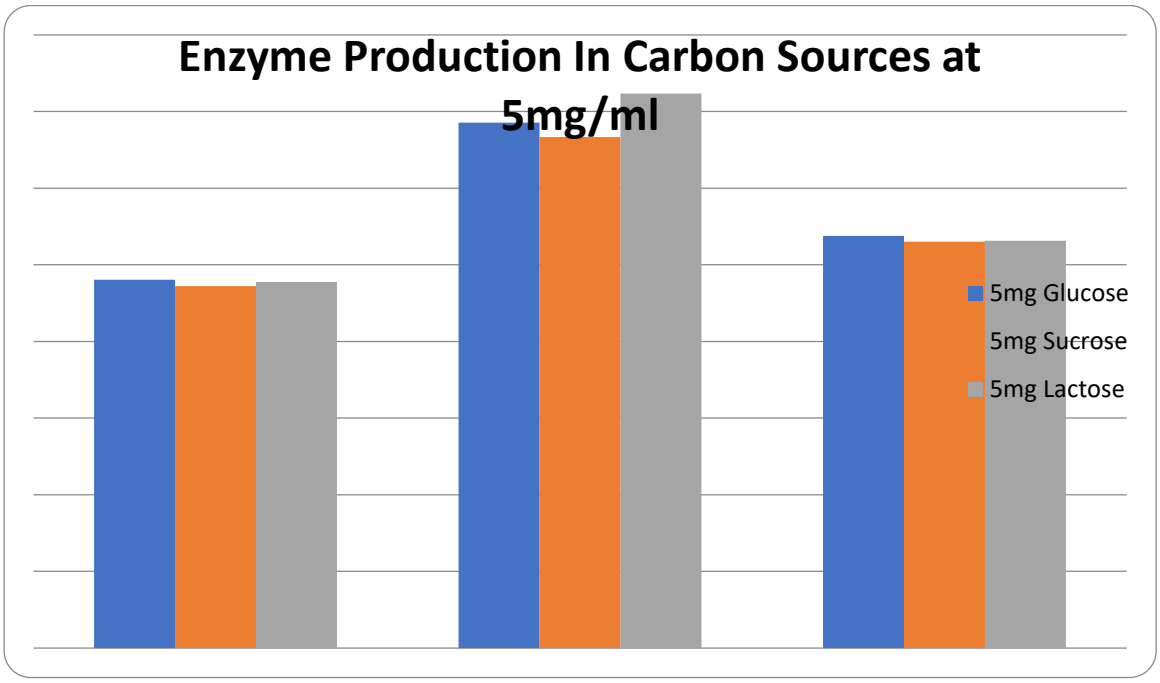
Sample	**Abs 420nm**	**Abs 420nm**	**Abs 420nm**	**Carbon Source**	**concentration**	**Activity**
Blank	0.002	0.003	0.001		2	
Isolate PT1	0.821	0.821	0.821		2	45.61
Isolate PT2	1.167	1.167	1.167	**Glucose**	2	64.83

Isolate PT3	0.917	0.917	0.917		2	50.94
Sample	**Abs 420nm**	**Abs 420nm**	**Abs 420nm**	**Carbon Source**	**Concentration Mg/ml**	**Activity**
Blank	0.002	0.003	0.001	**Glucose**	5	-
Isolate PT1	0.865	0.865	0.865		5	48.05
Isolate PT2	1.234	1.234	1.234		5	68.55
Isolate PT3	0.968	0.968	0.968		5	53.77

Figure.3.



Graph. No. 1



Graph. No. 2

Discussion:

In the study to evaluate the potential of different carbon sources for the higher production of the enzyme beta galactosidase, our work included checking three carbon sources namely Lactose, Sucrose and Glucose. The two concentrations used for checking the production were 2 mg/ml and 5 mg/ml. The graph number 1 shows at 2mg/ml glucose was the best carbon source for PT2, which was also the overall best producer of the enzyme. However, lactose showed constantly better results for all the strains for the beta galactosidase production. The graph number 2 shows that at 5mg/ml there was jump into the production of beta galactosidase by PT2, whereas other isolates PT1 and PT3 show little rise into the production. This is a unique difference in PT2 behaviour, which produced enzyme at higher amount at 2mg/ml concentration of glucose as carbon source but showed even higher produce using lactose at 5mg/ml concentration.

In a study of different strains of lactic acid bacteria were assessed for their β-galactosidase productivity, and Lactobacillus acidophilus ATCC 4356 resulted with the highest production potential. Thereafter, optimal conditions for accomplishing high yields of β-galactosidase activity were determined. Maximal specific activity (1.01 IU mL–1) was accomplished after 2 days shake flask culture fermentation (150 rpm) at 37 °C, with modified Man Rogosa Sharpe culture broth using lactose (2.5%) as sole carbon source (**Milica Carevic et al., 2015**). Even though this study was conducted on bacterial strain producing beta galactosidase, our study with the fungal isolates also shows some similarities with the findings. The different molecules play role of inducers and inhibitors for the switching on and off of particular genes. Here, the above findings along with our findings show that lactose is responsible for inducing the production of higher quantity of beta galactosidase enzyme in its presence in the production media as carbon source. This can be taken

as standard and combined with the paddy or wheat straws as substrate for the production of beta galactosidase at commercial levels.

In a study on banana isolated Aspergillus alliaceus when immobilized the team of **S. Sen., 2012** found that glucose was found to inhibit the enzyme activity of thermostable beta galactosidase enzyme produced by it. However, it was also observed that the immobilized enzymes retained only 43% of the beta galactosidase activity as compared to partially purified enzyme. And on storage there were no significant loss into the enzyme activity when kept at 4ºC for 28 days. The immobilized enzymes retained 90% of initial activity even after been used 4 times. When compared with the enzyme isolated from our fungal isolate PT2, which also has thermostability and greater activity, we believe it is also a great candidate for immobilization. But the initial loss in the activity during the immobilization may happen. This could be due to less permeability of the immobilizing agent. Also, due to reduction in the activity there might be loss of activity due to glucose molecule which may have hindered entry through the immobilized beads or substrate may not be reaching ample quantity for the enzyme to act on it.

In a study on T. viride, the effect of seven different carbon sources and six different nitrogen sources on lactase production was investigated. Lactose, which is the substrate in the media, was replaced with 1% glucose, galactose, sucrose, maltose, fructose and xylan separately. Finally, in order to investigate the additional glucose in the culture media on lactase synthesis, two parallel experiment sets were used. The first set was the basic media and the other was containing 0.1% glucose as carbon sources. Consequently, T.viride ATCC 32098 were grown on lactose and lactose plus glucose as carbon sources for comparison purposes. It was observed that activity is low in all carbon sources except lactose. Under circumstances where there exist easily metabolized carbon sources, the microorganism prefers this carbon source and may not be able to realize lactase responsible for lactose hydrolysis at high rates. This can also be explained by the suppressing effect of glucose on lactase synthesis. It is probable that sugars other than lactose also cause suppression in a similar manner. In addition, given xylan's more complex carbon source, media containing xylan also demonstrate low lactase activity. When growth in this medium is studied, the existence of high rates of growth indicates to the probability that the microorganism may have tended toward the synthesis of enzymes it could have utilized for this carbon source (**Işıl Seyis et al., 2007**). In our study the concentration of the carbon sources was 2% and 5% respectively. Even though we did take only 3 different carbon sources, the results obtained were similar to that found in the above study. The fungal isolates after biochemical and molecular analysis revealed to be an Aspergillus strain. Both T. viride and Aspergillus have have property to utilize complex sources of carbon or sugar to derive their energy. We have been also testing our isolates against the complex carbon sources like paddy straw and wheat straw. The purpose of these tests is to study utilization of our fungal isolates into naturally degrading these agro based waste materials and turn them into something useful for the mankind while protecting the nature against further pollution. Unlike the T. viride in the previous study, our isolate PT2 showed no inhibition against glucose as carbon source. May be the fungal strain isolated by us has any alternate metabolic mechanism that helps

it to even utilize glucose and not lose the enzyme production and activity. Further study can be conducted to determine this unique property and understand the mechanism behind its resistance.

Conclusion: There are multiple options available out there that can be supplemented as the source of carbon or any other nutrient for the fungus to grow. However, the above study is clear indicative that addition of lactose as the carbon source is better option to fulfill our requirements of beta galactosidase production. In a case of immobilization there may be reduction in the enzyme activity but it is still a matter of study for our isolates on immobilization. In addition to this the comparison with other fungal strains likes T. viride shows similar pattern into effect of different carbon sources including lactose. But our isolate has not shown any inhibition in enzyme activity in the presence of glucose. A small study using solid state fermentation of our isolate with paddy straw a substrate for the growth and production of the enzyme showed positive results. This indicates the ability of our isolate to utilize complex carbon source, which can be used to abate many environmental pollutants from the soil.

References:

1. Milica Carevic, Maja Vukašinović-Sekulić , Sanja Grbavčić , Marija Stojanović , Mladen Mihailović , Aleksandra Dimitrijević , Dejan Bezbradica (**2015**). Optimization of β-galactosidase production from lactic acid bacteria. Hem. Ind. 69 (3) 305–312.
2. Neidhardt, F. C., Ingraham, J. L. & Schaechter, M. (**1990**). *Physiology of the Bacterial Cell: a Molecular Approach* (Sinauer, Sunderland).
3. Schönheit, P., Buckel, W. & Martin, W. F. (**2016**). On the origin of heterotrophy. *Trends Microbiol.* **24**, 12–25.
4. Lehninger, A. L., Nelson, D. L. & Cox, M. M. (**2008**). *Lehninger Principles of Biochemistry* 5th edn. (W.H. Freeman, New York).
5. Randy A. Schemidt, Jun Qu, James R. Williams, and William S. A. Brusilow (**1998**). Effects of Carbon Source on Expression of F_o Genes and on the Stoichiometry of the c Subunit in the F_1F_o ATPase of *Escherichia coli.* J Bacteriol. 180(12): 3205–3208.
6. P.S. Panesar, S. Kumari, R. Panesar, (**2010**). Potential Applications of Immobilized β-Galactosidase in Food Processing Industries, Enzyme Res., doi: 10.4061/2010/473137.
7. P.S. Panesar, R. Panesar, R.S. Singh, J.F. Kennedy, H.Kumar, (**2006**). Microbial production, immobilization and applications of β-D-galactosidase, J. Chem. Technol. Biot. **81,** 530–543.
8. Q. Husain (**2010**). *β* Galactosidases and their potential applications: a review, Crit. Rev. Biotechnol. **30,** 41–62.
9. M.R. Kosseva, P.S. Panesar, G. Kaur, J.F. Kennedy (**2009**). Use of immobilised biocatalysts in the processing of cheese whey, Int. J. Biol. Macromol. **45,** 437–447.
10. S. Sen, L. Ray, P. Chattopadhyay (**2012**). Production, Purification, Immobilization, and Characterization of a Thermostable β-Galactosidase from *Aspergillus alliaceus*, Appl. Biochem. Biotech. **167,** 1938–1953.

11. Ansari SA, Satar R (**2012**). Recombinant β-galactosidases- past, present and future: A mini Review. J. Mol. Catal. B: Enzym. 81:1-6.
12. Işıl Seyis, Nilüfer Aksöz (**2007**). Effect of Some Medium Components on Lactase Production from T. Viride ATCC 32098. Hacettepe J. Biol. & Chem., 35 (2), 89-92.
13. Fantes, P.A. and Roberts, C.F. (**1973**). β-galactosidase activity and lactose utilization in Aspergillus nidulans, J Gen Microbiol, 77, 471-486.
14. Deshpande, M.S., Deopurkar, R.L. and Rale, V.B. (**1989**). Beta-galactosidase from Auerobasidium pullulans, Lett Appl Microbiol, 9, 21-24.
15. Harper, W.J. Milk components and their characteristics, Dairy Technology and Engineering, 2nd Ed., (**1981**). Harper W. J. and Hall C. W.(eds). Avi Publishing Company, Inc. Connecticut, 18-75.
16. Apartin, C. and Ronco, A. (**2001**). Development of a free beta-galactosidase in vitro test for the assessment of heavy metal toxicity. Environ Toxicol Water Qual, 16, 117-120.
17. Hatzinikolaou, D.G., Katsifas, E., Mamma, D., Karagouni, A.D., Christakopoulos, P. and Kekos, D. (**2005**). Modeling of the simultaneous hydrolysis–ultrafiltration of whey permeate by a thermostable β-galactosidase from Aspergillus niger, Biochem Eng J, 24(2), 161-172.
18. D. J. Mukesh Kumar, M. Sudha, S. Devika, M. D. Balakumaran, M. Ravi Kumar, P. T. Kalaichelvan (**2012**). Production and Optimization of β-galactosidase by Bacillus Sp. MPTK 121, Isolated from Dairy Plant Soil. Annals of Biological Research, 3 (4):1712-1718.
19. Nurullah AKCAN (**2011**) High level production of extracellular β-galactosidase from Bacillus licheniformis ATCC 12759 in submerged fermentation. African Journal of Microbiology Research Vol. 5(26), pp. 4615-4621.
20. S. Nizamuddin, A. Sridevi and G. Narasimha (**2008**). Production of β-galactosidase by Aspergillus oryzae in solid-state fermentation. African Journal of Biotechnology Vol. 7 (8), pp. 1096-1100.
21. Raquel Dall'Agnol Martarello, Luana Cunha, Samuel Leite Cardoso, Marcela Medeiros de Freitas, Damaris Silveira, Yris Maria Fonseca-Bazzo , Mauricio Homem-de-Mello, Edivaldo Ximenes Ferreira Filho and Pérola Oliveira Magalhães (**2019**). Optimization and partial purifcation of beta-galactosidase production by Aspergillus niger isolated from Brazilian soils using soybean residue. AMB Expr 9:81.
22. Basil JM (**1981**). Production of extra cellular lactase from Fusarium moniliforme. Appl. Microbial. Biotechnol. 13: 161-164.
23. Konsoula Z, Liakopoulou-Kyriakides M (**2007**). Co-production of αamylase and β-galactosidase by Bacillus subtilis in complex organic substrates. Bioresource Technol., 98: 150-157.