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Comparative Study Between *Aspergillus niger* and *Saccharomyces carlsbergensis* for the Production and Characterization of Invertase by Submerged Fermentation Technique

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Aspergillus niger and Saccharomyces carlsbergensis **were grown in submerged fermentation systems with sucrose concentration at 100 g/ liter and varying concentration of molasses in Basic Mineral Medium (BMM) for production and characterization of invertase by *Aspergillus niger* and *Saccharomyces carlsbergensis*. As compare with *Aspergillus* niger the *Saccharomyces carlsbergensis* produce exceptionally large amount of extra cellular invertase in sucrose and varying conc. of molasses. The optimum temperature of inveratse for *Aspergillus niger* is 40° C and to that of *Saccharomyces carlsbergensis* is 50 ° C. The optimum pH of invertase for *Aspergillus niger* was found to be 4.4 and to that of for *Saccharomyces carlsbergensis* is 4.8 . The activity of this enzyme under these conditions is 600 μg of reducing sugar /1 min/1 ml of enzymes for *Aspergillus niger* and to that of *Saccharomyces carlsbergensis* is 5040μg of reducing sugar/1 min/1 ml of enzyme. For these two i.e *Aspergillus niger* and *Saccharomyces carlsbergensis* the specific activity was found to be 2.25 and 20.16 respectively. Immobilization was also done for getting immobilized cells activity. And finally gel electrophoresis is done to get separate protein bands.**

Keywords: Enzymes, *Aspergillus niger, Saccharomyces carlsbergensi,* Fermentation technique.

Invertase is glucosidase enzyme found in yeast. It catalyses the hydrolyses of sucrose to glucose and fructose. It is widely distributed in nature. *Saccharomyces fragilis*, *S. cerevisiae, Candiada utilis* are the richest source of enzyme invertase. Invertase is produced in industries from bakers yeast. (Robert G. Dworschack and Lynferd J. Wickerham).

Sucrose, commonly known as table sugar, is composed of an alpha-D-glucose molecule and a beta-D-fructose molecule linked by an alpha-1, 4-glycosidic bond. When this bond is cleaved in a hydrolysis reaction, an equimolar mixture of glucose and fructose is generated. The official name for invertase is beta-fructofuranosidase (EC3.2.1.26), Invertase is mainly used in the food (confectionery) industry

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where fructose is preferred over sucrose because it is sweeter and does not crystallize as easily. For health and taste reasons, its use in food industry requires that invertase be highly purified. (Nam Sun Wang et.al.1985) A wide range of microorganisms produce invertase and can, thus, utilize sucrose as a nutrient. Even within the same yeast culture, invertase exists in more than one form. In contrary to most other enzymes, invertase exhibits relatively high activity over a broad range of pH (3.5—5.5), with the optimum near pH=4.5. The enzyme activity reaches a maximum at about 55ºC. The Michaelis-Menten values of various enzymes vary widely, but for most enzymes K_m is between 2 mM and 5 mM(Nam Sun Wang *et. al.*, 1985) The Michaelis-Menten value for the free enzyme is typically approx. 30 mM. In this experiment, the kinetics of invertase is investigated with the method of initial reaction rates. The enzyme-substrate mixture is allowed to react for a specified amount of time. The rate of reaction can be easily monitored by measuring the amount of reaction products, i.e., an equimolar mixture of glucose and fructose. The amount of reducing sugars produced was determined colorimetrically Miller GL (1959) with the dinitrosalicylic acid (DNSA). The task is made easier since the DNSA reagent does not react with sucrose. The optimum temperature, pH, and specific enzyme activity has also been studied. And comparative study was done between *Aspergillus niger* and *Saccharomyces carlsbergensis.*

MATERIAL AND METHODS

For Submerged fermentation two 500ml flasks with 250ml of BMM medium (Basic Mineral Medium) was inoculated separately with *Aspergillus niger* and *S. carlsbergensis* supplemented with 100 g sucrose per liter. Submerged fermentation was done in 500ml flasks filled with 250ml of BMM medium at 30^0 C in an orbital shaker at 200rpm. Enzyme activity and protein content were determined at every 24, 48, 72, and 96 hours respectively of these flaks. From this information specific activity at every 24, 48, 72, and 96 hours were determined.

Determination of optimum pH

a. Invertase activity was assayed for both *Aspergillus niger* and *Saccharomyces carlsbergensis* at different pH values using sodium acetate buffer at different pH values i.e. 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6
b. For determination of pH optima of enzyme, reaction mixture was made as as shown in table. pH optima was carried out for both *Aspergillus niger* and *Saccharomyces carlsbergensis*

Determination of optimum temperature:

Invertase activity was assayed for both *Aspergillus niger and Saccharomyces carlsbergensis* at different temperature values using sodium acetate buffer at different temp. values i.e. 4°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C at pH 4.8. For determination of optimum temperature of enzyme, reaction mixture was made as following addition table. Temperature optima was carried out for both *Aspergillus niger and Saccharomyces carlsbergensis.*

Effect of carbon sources

Different molasses concentration were checked for both these strains. For both strains molasses concentration in the range 50mg, 100mg, 500mg, 1gm were checked. And the concentration giving highest activity for both strains was recorded.

Immobilization

Immobilization of both enzymes at 72 hours with beads of *Aspergillus niger* and *Saccharomyces carlsbergensis* were done. For the immobilization, 5 gm of yeast cells from respected species were collected and to this there is addition of 10ml of 4% sodium alginate (keep for overnight) and mixing is done with stirrer. Taking this mixture and by adding into buffer containing 2% $Cacl_2$ with constant stirring, drop by drop due to which uniform beads are formed. And results were recorded. This immobilized activity was also checked for continuous biomass use as like this a column was packed with medium (BMM) and beads of both *Aspergillus niger* and *Saccharomyces carlsbergensis* and there respective activity was checked for various time periods i.e. after 24, 48, 72.

RESULTS AND DISCUSSION

Invertase producing organisms i.e. *Aspergillus niger* and *Saccharomyces carlsbergensis* are potent in nature and has immense potential to produce invertase enzyme by both solid and submerged fermentation. It has wide utility in various applications. Some industrial strains of *Aspergillus niger and Saccharomyces cerevisiae* produces extracellar and total invertase yields approaching to those of *Candida utilis***.** The ratio of extra cellular invertase produced in shake flask method was markedly higher for and *Saccharomyces carlsbergensis* than that of *Aspergillus niger* and other species**.** Perhaps this is because *Saccharomyces carlsbergensis* is less strongly fermentative than other species. There was great difference in total inveratse production between strains of *Aspergillus niger* and *Saccharomyces carlsbergensi*. Thus it has greater advance in all industries and as well as in research project so I have done this work.

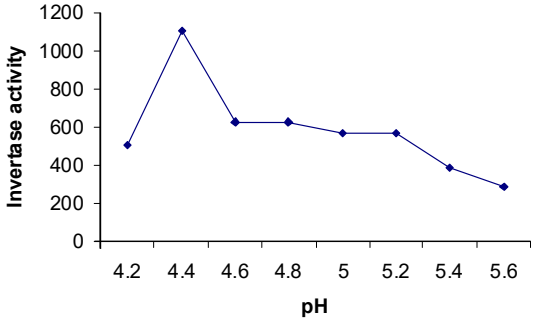
Table 1. Optimum pH for invertase production by *Saccharomyces carlsbergensis* and *Aspergillus niger*

Enzyme	Enzyme activity µg/ml of enzyme /min.	Protein content/ml	Specific activity
Aspergillus niger	100	266	2.25
Saccharomyces carlsbergensis	840	300	20.16

Table 2. *Aspergillus niger* Optimum pH

PH	µgm of reducing sugar formed /0.1ml of enzyme	Invertase activity µgm/ml of sugar/ml of enzyme/min.
4.2	85	510
4.4	185	1110
4.6	105	630
4.8	105	630
5.0	95	570
5.2	95	570
5.4	85	510
5.6	85	390

Thus the optimum PH for *Aspergillus niger* is 4.4



Previous work on invertase production and characterization was done by scientist "Robert G. Dworschack and Lynferd J. Wickerham." They found that some strains of *Candida utilis* produce exceptionally large amounts of extra cellular and total invertase. Strain Y-900 of *C. utilis* produces high yields whether the carbon source is sucrose, glucose, maltose, or xylose and still higher yields with lactic acid, glycerol, and ethyl alcohol. Approximately 20 to 30% of the total invertase of *C. utilis* is extracellular. Strains of *Saccharomyces cerevisiae* and *Aspergillus niger*

Table 3. Optimum pH *for Saccharomyces carlsbergensis*

PH	µgm of reducing sugar formed /0.1ml of enzyme	Invertase activity µgm/ml of sugar/ml of enzyme/min.
4.2	65	390
4.4	85	570
4.6	85	510
4.8	125	750
5.0	15	630
5.2	95	570
5.4	95	570
5.6	75	450

Thus the optimum PH for *Saccharomyces carlsbergensis* is 4.8

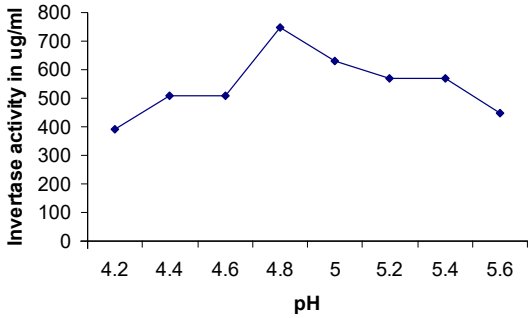


Table 4 Optimum temperature for *Aspergillus niger*

PH	µgm of reducing sugar formed /0.1ml of rex. Mix	Invertase activity µgm/ml of sugar/ml of enzyme/min.
0	15	90
10	50	300
20	74	444
30	95	570
40	100	600
50	75	450
60	60	360
70	60	360
80	15	90

Thus the optimum temperature for *Aspergillus niger* is 40ºC

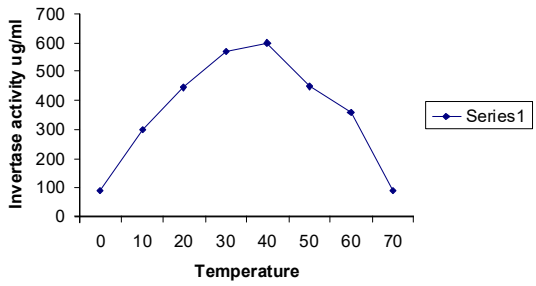


Table 5. Optimum temperature *Saccharomyces carlsbergensis*

PH	µgm of reducing sugar formed /0.1ml of rex. Mix	Invertase activity µgm/ml of sugar/ml of enzyme/min.
0	10	60
10	25	150
20	45	270
30	60	360
40	75	450
50	80	480
60	60	360
70	25	150
80	20	90

Thus the optimum temperature for *Saccharomyces carlsbergensis*is 50ºC

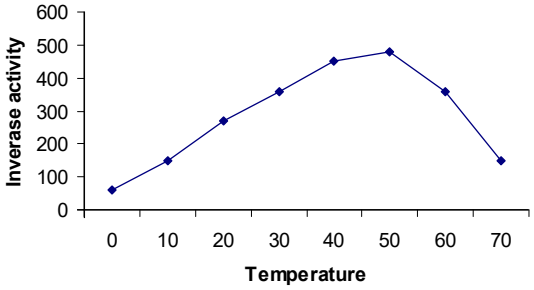


Table 6. Highest enzyme activity was observed at molasses concentration 1gm at 48 hours for *Aspergillus niger* than any other concentration

S. No	50mg	100mg	500 mg	1 gm
Test	0.04	0.06	0.08	0.18
E.C	0.00	0.02	0.02	0.00
S.C	0.02	0.00	0.00	0.02

Table 7. Highest enzyme activity was observed at molasses concentration 1gm at 72 hours for *S. carlsbergensis* than any other concentration

S. No	50mg	100mg	500 mg	1 gm
Test	0.10	0.14	0.22	0.38
E.C	0.02	0.00	0.04	0.05
S.C	0.02	0.04	0.02	0.00

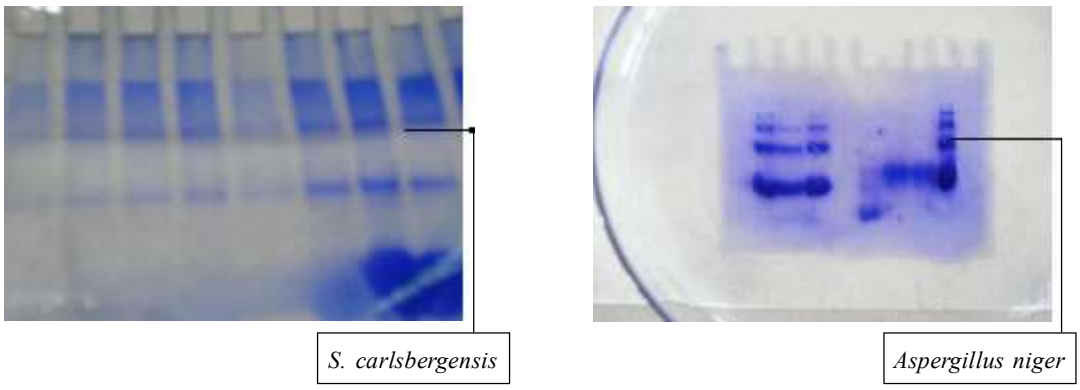


Fig. 1. Protein bands after gel electrophoresis

are generally inferior to *C. utilis* in production of extracellular and total invertase, the difference being accentuated in shaken cultures.

CONCLUSION

The comparative survey was made between *Aspergillus niger* and *Saccharomyces carlsbergensis* but it seems to be clear that *S. carlsbergensis* produces large amount of extra cellular invertase in shake flask method. Some industrial strains like *Saccharomyces cerevisiae* produces extracellular and total invertase yields approaching those of Candida utilis. There is great difference in total invertase production between strains in both *Saccharomyces cerevisiae* and *Candida utilis*. But our conclusion is that *S. carlsbergensis* yields large amount of invertase than other species**.** On an average total protein content, specific enzyme activity, and production yield was superior for *S. carlsbergensis* than other species.

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