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COMPARATIVE ANALYSIS IN THE PRODUCTION OF AN INVERTASE FROM JUICE ISOLATES

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ABSTRACT

Invertase enzymes are produced mainly by plants, some filamentous fungi, yeast and many other microorganisms which find applications in food industries, confectionaries, pharmaceuticals etc. The present work deals with the production of Invertase by *Bacterial sp.*, and isolated from soil samples in solid state fermentation using carrot peel waste as substrate. Enzyme activity was checked using Sucrose and assay was carried out by DNS method. The results were comparatively analysed between wild strain and mutated strain. It was found that the enzyme production from UV mutated strain is higher as compare to EtBr mutated strain & wild strain.

Key words: Invertase, Beta-fructofuranosidase, Metabolism, Mutation, Ethidium bromide.

INTRODUCTION

Invertase or official name beta-fructofuranosidase is a commercially important enzyme used for the hydrolysis of sucrose. This hydrolysis of sucrose yields an equimolar mixture of glucose and fructose, known as invert sugar. Invert sugar is sometimes referred to as artificial honey since its composition and properties are nearly same **[1]**.

Enzymatic invert sugar is safe as it also an absolute health-friendly sweetener. So, the enzyme invertase is employed for the hydrolysis of sucrose and fructose. Nowadays, fructose is a sugar which can be consumed by diabetic patients **[2]**. This because its metabolism which is insulin independent. Also, invert sugar is 28 to 30% sweeter than table sugar so the amount of sugar required for particularly degree of sweetness is also reduced. Finally, the enzymatic invert sugar does not involve the use of any chemicals or acids unlike conventional acid- hydrolyzed invert syrup. Hence, it is completely healthy sweetener **[3-7].**

Traditionally, invertase usually produced on site by autolysing yeast cells and is sold either as a clear liquid or as a powder that can be dissolved in water **[8]**. Its name refers to its ability to change the direction of optical rotation of sucrose solution as a result of hydrolysis to glucose and fructose **[9,10]** According to Verma and Dubey (2001) invertase is a group of ubiquitous enzymes with different pH optimum and subcellular localization. Invert sugar is frequently used in commercial baking and candy recipes because it keeps baked goods moist for longer periods of time **[11,12]**. The invertase itself should be stored in the refrigerator for longevity. Optimum conditions for invertase are pH 3.0 to 6.0 and temperature 40°C to 80°C **[13]**.

METHODOLOGY

Sample collection:

The juice samples were collected from different locations which consists of enough content of sucrose in the juice. The samples were collected from juice corners **[14]**.

Isolation of Invertase producing bacteria:

The samples were serially diluted in normal saline and then spread on sterilized nutrient agar plates. Further pure cultures were prepared by selecting the colonies on the basis different morphological parameters **[15,16]**.

Screening of Invertase producing bacteria:

The screening was carried by observing the growth of bacterial cultures and the enzyme production assay was analyzed by performing the DNS assay **[17]** and Lowrys assay **[18]**.

Strain identification:

The identification of culture was carried out by performing the staining tests and biochemical tests following Bergys manual **[16]**.

Strain improvement:

Strain improvement was carried out by performing the physical (UV rays) and chemical (EtBr) mutation. Further these cultures were screened for Invertase production **[19]**.

Fermentation & downstream processing:

The cultures were inoculated in sterilized minimal salt media supplemented with 1% sucrose and incubated for one week further the purification of Invertase was carried out by performing the salt precipitation and dialysis. Then the enzyme activity was checked **[20].**

RESULTS

Isolation of bacteria from juice:

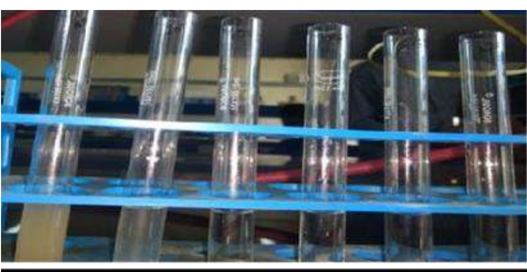


Figure 1: Isolated cultures from juice samples.

Total 17 cultures were isolated from the juice samples after serial dilution and spreading.

Table 1: *Primary & secondary screening of Invertase producing bacteria*

CULTURE NO.	PRIMARY SCREENING	SECONDARY SCREENING
1	++	-
2	++	-
3	+	-
4	+	-
5	-	-
6	-	-
7	**+++**	**+++**
8	+	++
9	+	+
10	+	+
11	++	+
12	+	-
13	+	-
14	_	-
15	_	-
16	_	+
17	_	+

UV rays mutation:

Table 2: Four different time interval was selected for the mutation.

Sr. No.	Sample	OD at 540 nm	OD at 680 nm
1	**Control**	0.23	0.29
2	**UV 2 min**	0.34	0.03
3	**UV 4 min**	0.50	0.98
4	**UV 6 min**	0.40	0.25
5	**UV 8 min**	0.61	0.65

Figure 2: Four different time interval was selected for the mutation.

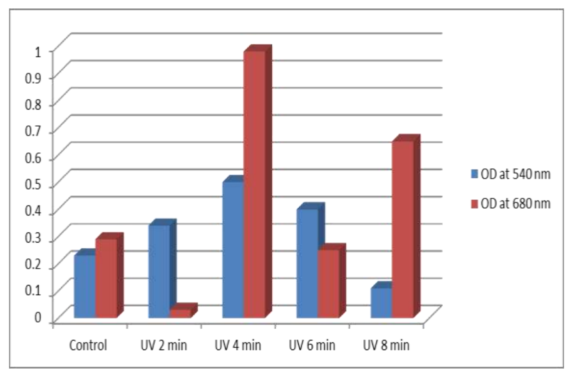


Figure 3: Graphical analysis of four different time interval was selected for the mutation.

Ethidium bromide mutation:

Table 3: Six different concentrations of ethidium bromide were selected for the mutation.

Sr. No.	Sample	OD at 540 nm	OD at 680 nm
1	**Control**	0.24	0.29
2	**2 µl**	0.37	0.63
3	**4 µl**	0.50	0.21
4	**6 µl**	0.61	0.69
5	**8 µl**	0.25	0.14
6	**10 µl**	0.01	0.01
7	**12 µl**	0.50	0.50

Figure 4: Six different concentrations of ethidium bromide were selected for the mutation.

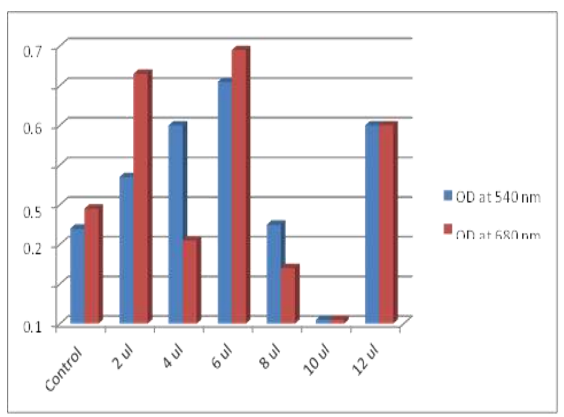


Figure 5: Six different concentrations of ethidium bromide were selected for the mutation.

Estimation of enzyme by DNS and Lowrys assay:

Table 4: Enzyme assay by DNS and Lowrys method.

Sr. No.	Sample	OD at 540 nm	OD at 680 nm
Crude enzymes			
1	Wild strain	0.15	0.4
2	UV 4 min mutated strain	0.72	0.8
3	EtBr 6 µl mutated strain	0.32	0.6
Pure enzyme			
1	Wild strain	0.13	0.32
2	UV 4 min mutated strain	0.62	0.71
3	EtBr 6 µl mutated strain	0.52	0.47

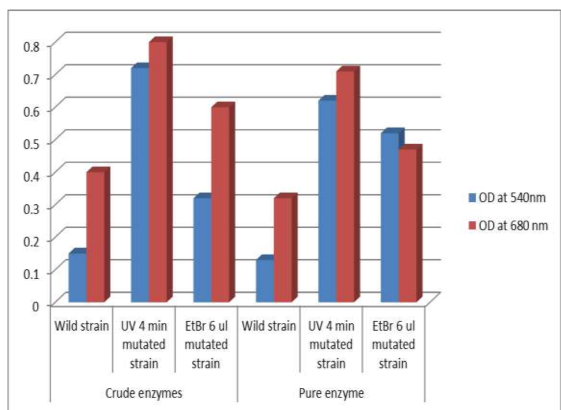
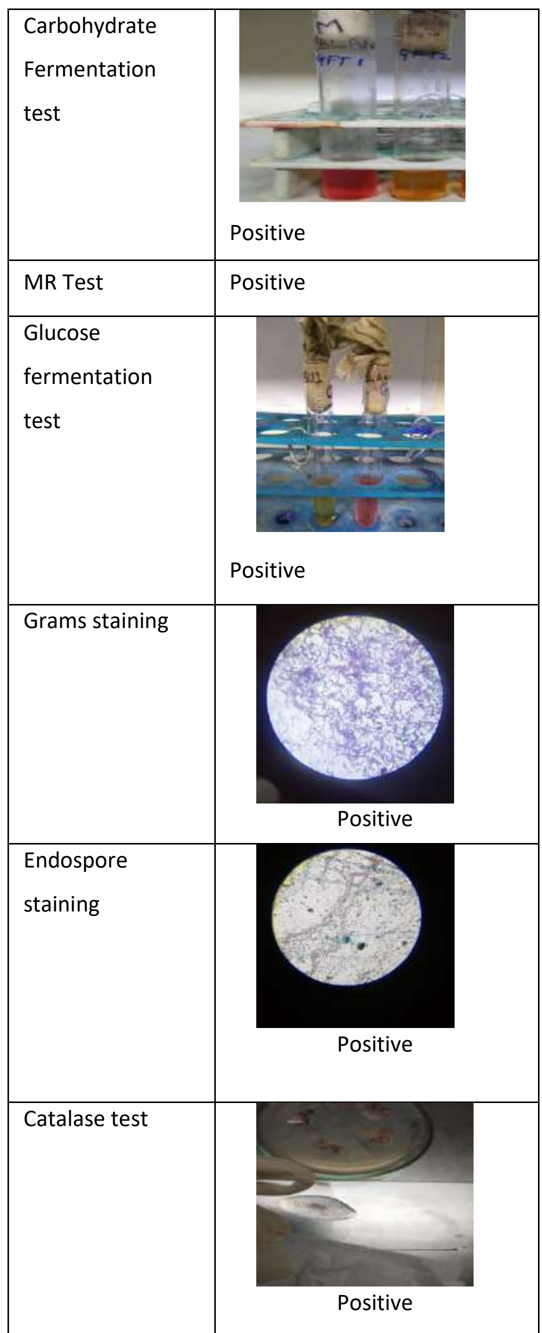


Figure 6: Graphical representation of enzyme estimation.

Strain Identification:

Tests	Results
Mannitol test	Positive
VP Test	Negative



Tests	Results
Carbohydrate Fermentation test	Positive
MR Test	Positive
Glucose fermentation test	Positive
Grams staining	Positive
Endospore staining	Positive
Catalase test	Positive

DISCUSSION AND CONCLUSION

The samples were serially diluted in 0.85% NaCl solution, further the diluted samples were spread on Nutrient agar plates and then on the basis of various morphological parameters the cμltures were converted to pure cμltures and streaked on fresh nutrient agar plates and then screened for Invertase on minimal salt media supplemented with sucrose. The positive cμlture was identified by performing the various biochemical methods. It was found the cμlture belongs to *Bacillus cerus.*

Invertase enzyme was produced by bacteria and for better production media for bacteria was optimized and strain improvement was done by physical and chemical mutation methods. In physical mutation UV rays exposure was carried out and in chemical mutation the cμltures were treated with ethidium bromide which act as a carcinogen.

All three strains were allowed for fermentation by shake flask method & batch fermentation and the purification of enzyme was carried out by salt precipitation and dialysis.

By performing the comparative analysis on the enzyme production on the basis of the absorbance it was found that the UV mutated 4 min cμlture shows maximum production as compare to EtBr 6 µl mutated strain and wild strain.

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