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ISOLATION AND EXTRACELLULAR SCREENING OF GLUCOSE ISOMERASE PRODUCING BACTERIA ISOLATED FROM AGRICULTURE SOIL

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ABSTRACT

Microbial enzymes have proven their value in bio-industries such as food, animal feed, leather, textiles as well as in bioconversions and bioremediations. Glucose isomerase (GI) catalyzes the reversible isomerization of glucose to fructose *in-vitro* and that of xylose to xylulose *in-vivo*. The enzyme has the largest market in the food industry because of its application in production of high fructose corn syrup. Naturally present many microorganisms have the ability to produce glucose isomerase enzyme. The present study was aimed to screen the bacterial isolates for extracellular glucose isomerase activity. Agriculture soil samples were collected from Badgaon and Bari, Udaipur. Isolation was done on nutrient agar plate using pour plate method. Screening of the isolates for glucose isomerase production was done on screening medium containing 1% glucose and the enzyme activity was tested with Seliwanoff's reagent. The observation was made to check the substrate utilized and formation of cherry red colour which indicates the presence of fructose. A total of 20 numbers isolates were recovered on nutrient agar. Out of 20, only five isolates showed cherry red color on addition of Seliwanoff's reagent which indicates the production of fructose. The isolates that showed glucose isomerase activity can be further explored for quantitative analysis for the production of glucose isomerase and may be proved as possible candidates for commercial production of high fructose corn syrup.

KEYWORDS: Glucose isomerase (GI), enzymes, bioconversion, Seliwanoff's reagent, HFCS.

INTRODUCTION

Enzymes are stimulating influence proteins and are responsible for a numeral of necessary chemical transformations, and play significant roles in food industry (Gupta, 2016). Glucose isomerase (GI) plays a vital role in the food industry as it serves as a catalyst for the interconversion of glucose to fructose. Glucose isomerase (also known as xylose isomerase, EC.5.3.1.5) catalyzes the reversible isomerization of D-glucose and D-xylose to D-fructose and D-xylulose, *in vitro* and *in vivo* respectively (Bhosale *et al.*, 1996). In isomerization, the intra-molecular rearrangements of isomers occur between keto- and aldo-sugars (Rose *et al.*, 1969). Conversion of xylose to xylulose helps as nutritional requirement in saprophytic bacteria that succeed on decaying plant substantial and also benefits in the bioconversion of hemicellulose to ethanol. Isomerization of glucose to fructose is of viable significance in the production of high fructose corn syrup (Nwokoro, 2015).

Microorganisms are potential producers of enzymes useful for the food industry. Microbial enzymes are notorious to be superior enzymes obtained from different microorganisms, particularly for uses in industries on marketable scales (Singh *et al.*, 2016). Glucose isomerase (GI), also known as D-xylose isomerase, is identical important water soluble enzyme normally applied in drinks and several products in the food industry. Glucose isomerase is an essential process for the industrial production of high fructose corn syrup (HFCS) and it is main sweetener for component in many soft drinks and food. Glucose isomerase has gained much attention due to its potential health and medical aids for the production of infrequent monosaccharides such as L-glucose, L-fructose, L-ribose, L-lyxose, D-allose and L-galactose (Song *et al.*, 2011; Kluskens *et al.*, 2010). The growth in food, healthcare, fuel ethanol, animal feed is prompting the demand for GI (Bhasin and Modi, 2012). The present study was aimed to screened extracellular glucose isomerase producing bacteria for production of fructose which can be used for high fructose corn syrup production. Therefore, the research put frontward to isolate some bacterial species from agricultural soil and screening of extracellular glucose isomerase to develop a bioprocess for the production of fructose.

MATERIALS AND METHODS

1. Chemicals

Chemicals which were used during this study were purchased from Himedia and Sigma-Aldrich India.

2. Collection of agricultural soil samples

For the isolation of glucose isomerase producing bacteria, two agricultural soil samples were collected from Bari 24.6079' latitude & 73.635' longitude and Badgaon, 24.6366' & 73.6801' longitude, district Udaipur, Rajasthan, India (Geographical map shows in Figure 1(a) and (b). The samples were collected in sterile polythene bags and transferred to the laboratory. The temperature of the soil samples during samples collection was 30˚C. The pH of the samples was observed to be7.

3. Isolation of bacteria from agricultural soil samples

Isolation was done on nutrient agar using pour plate method. The samples were serially diluted and an aliquot of 100µl of each dilution (10^{-1} to 10^{-10}) was then transferred to petriplates containing 30 ml nutrient agar. The petridishes were then inverted and incubated at 37˚C, for 48 h. The plates were further purified by streak plate method. The pure cultures were maintained on nutrient agar slants and nutrient broth for further use.

4. Screening for extracellular glucose isomerase producing bacteria

The screening for producing extracellular glucose isomerase was done by submerged fermentation using Seliwanoff's reagent. The bacterial isolates were inoculated into a 150 ml conical flask containing 50 ml of culture medium (Peptone 1%, Yeast extract 0.5%, K_2HPO_4 0.3%, $MgSO_4$. $7H_20$- 0.1%, and with glucose 1%, distilled water 50 ml, pH 7.0) followed by incubating at 37ºC in a incubator shaker (180rpm) for 24 hours method describe by Nobelsurya *et al.*, 2011.The supernatant was screened for the production of extracellular glucose isomerase using Seliwanoff's reagent. The observation was recorded to check the utilization of glucose as substrate and development of cherry red colour which indicate production of fructose.

Figure: 1 Sample sites (a) Bari | **(b) Badgaon**

RESULTS AND DISCUSSION

For the present study the soil samples were collected from agricultural land of Bari and Badgaon, Udaipur, Rajasthan, India. Nutrient agar medium was used for the isolation of bacteria. A total of 20 bacterial isolates were isolated from the two agricultural soil samples. All the 20 isolates were screened for presence of extracellular glucose isomerase. Out of 20 isolates five isolates (Figure 2) found to be positive for glucose isomerase production as indicated by cherry red color formation by adding Seliwanoff's reagent (Figure 3).

Nobelsurya *et al.*, 2011 reported that bacterial isolate *Enterobacter agglomerans* was found positive for glucose isomerase production by adding Seliwanoff's reagent as showed cherry red color formation. The present study also interrelated with research work done by Sathya and Ushadevi, 2014, who reported that *Streptomyces* species showed positive reaction by development of cherry red colour on addition of Seliwanoff's reagent.

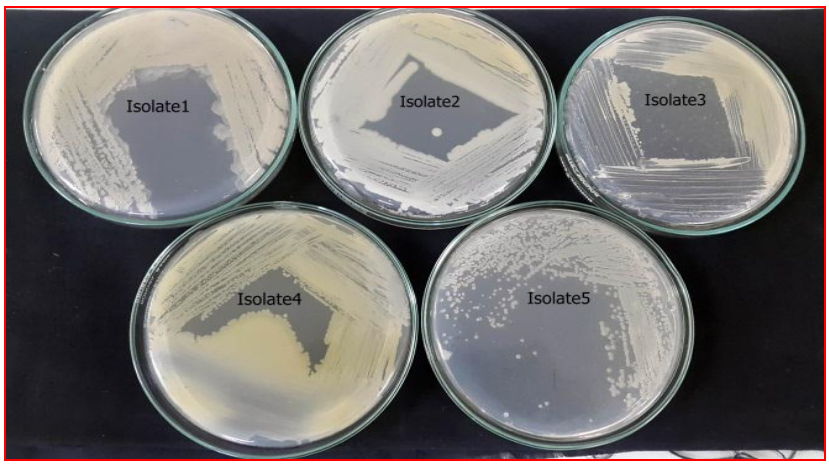

Figure 2: Pure cultures of the five isolates which showed glucose isomerase activity.

Figure 3: Extracellular glucose isomerase activity of the isolates showing cherry red color on addition of Seliwanoff's reagent.

CONCLUSION

Microorganisms found in environmental soil habitats adapt to conditions that may have great biotechnological potential. Microorganisms produce potential enzymes which have beneficial characteristics for industrial processes. The five bacterial strains that showed glucose isomerase activity may be used for commercial production of glucose isomerase can be further used for HFCS production. HFCS has many advantages compared to sucrose that make it attractive to food manufacturers.

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