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Isolation and screening of glucose isomerase producing marine *Streptomyces* species for fructose production

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ABSTRACT

The present study is to isolate and screening of glucose isomerase from marine Streptomyces species for industrial application of fructose. Soil samples were collected from Muthupet mangroves, Tamilnadu, India. Marine Streptomyces has emerged as an in exhaustive treasure for a wide range of enzymes like glucose isomerase. Glucose isomerase screening was performed by three different methods such as plate assay using xylose containing peptone yeast agar medium, fructose estimation by seliwanoff's test and isomerization of glucose to fructose was detected by thin layer chromatography. Streptomyces species were grown on peptone yeast extract broth, the cell biomass was harvested then disrupted by ultrasonic disintegrator and centrifuged; cell free extracts were used for glucose isomerase activity. Thin layer chromatography separation provides a good result for isomerization of glucose to fructose by glucose isomerase. This finding proved numerous Streptomyces species has been produced glucose isomerase activity, particularly Streptomyces sp. RSU26 has been proved superior performing for glucose isomerase production and can be used for industrial application of fructose production.

Key words: *Streptomyces*, Intracellular, Glucose isomerase, TLC, Enzyme assay

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INTRODUCTION

Glucose isomerase/Xylose isomerase (EC. No. 5.3.1.5) is a commercially important enzyme that plays an essential role in microbial sugar metabolisms. The greatest market for glucose isomerase is in food industry; particularly catalyzes two important reactions such as reversible isomerization of D-glucose to D-fructose and D-xylose to D-xylulose for possible application to ethanol production from hemicelluloses [1]. The production of rare monosaccharides, such as L-glucose, L-fructose, L-ribose, L- lyxose L-galactose by glucose isomerase, recently has received much interest to potential health and medical benefits [2]. Glucose isomerase catalyzes the conversion of glucose to fructose, which has significant value in the production of High Fructose Corn Syrup (HFCS). The world market for glucose isomerase is US$1billion and about 100,000 tons of glucose isomerase is made annually in global scale [3, 4]. Thus, there is a great demand for glucose isomerase globally. Increasing demand for refined sugar, coupled with high cost of production and awareness of the adverse effects of sucrose and invert sugar consumption on human health, fructose for acceptable sucrose substitutes. Fructose has the benefit an equal sweetener level, 10-20% cheaper than sucrose and less caloric, D-fructose plays an essential role in a diabetic sweetener because fructose is slowly reabsorbed by the stomach and does not pressure the glucose level in blood [5, 6]. HFCS is used as sweetener in soft drinks and other food products where fructose replaces beet and cane sugar. Technical advantage of HFCS is the good solubility of glucose and fructose compared to sucrose and the lesser tendency to crystallize in a wide range of food products [7].

Marine *Streptomyces* play a role in manufacture of many commercially important chemicals, including fructose production from glucose. Recent findings suggested enhancing the production of glucose isomerase in some marine actinobacteria; especially *Streptomyces* species are leading the enzyme market. Therefore, the research put forward to isolate some *Streptomyces* species from mangrove regions and screened intracellular glucose isomerase to develop a bioprocess for the production of fructose.

MATERIALS AND METHODS

Sampling site

The soil samples were collected from different region of Muthupet mangrove forest, Tamilnadu, India. The samples were collected at a depth within 15-25cm from the surface of the soil. The mangrove ecosystem is a largely unexplored source for actinobacteria with the potential to produce novel secondary metabolite.

Isolation of *Streptomyces*

Isolation of *Streptomyces* was performed by soil dilution plate technique using Starch casein agar supplemented with 90% sea water and antibiotics (Streptomycin and Gresiofulvin) was suggested [8]. The inoculated plates were incubated at 28ºC for two weeks, after incubation *Streptomyces* colonies were identified, based on the colony appearance, powdered, colored, smooth/rough with irregular/regular margins. After isolation, the pure *Streptomyces* cultures were preserved at 4^{o}C for further investigation [9].

Growth Characterization and identification of *Streptomyces*

Various approaches used for the identification of *Streptomyces* (Classical, biochemical)viz., colony morphology, spore chain ornamentation, aerial mass colour, substrate and aerial mycelium growth characteristics, reverse side pigmentation was described by Buchanan [10] Bergey's manual of determinative Bacteriology. Light Microscopy of *Streptomyces* was performed by coverslip culture technique [11]. The cover slips were removed after four days of incubation then dried and observed under the high power magnification. It was used to study the morphological features of spores, sporangia, aerial and substrate mycelium appearance. Bio chemical characterization of *Streptomyces* was determined using the method Shiriling and Gottlieb [12].

Screening for *Streptomyces* producing Glucose/Xylose Isomerase

Glucose isomerase activity was performed by following the method of Sapunova et al. [13]. *Streptomyces* were grown on Peptone-yeast agar with 0.01M of glucose as a substrate and incubated at 37^{o}C for four days. After incubation the plates were either untreated or treated with the reaction mixture a 0.1% 2, 3, 5-triphenyltetrazolium solution in 1 M NaOH at 35^{o}C for 1 min in the dark. Rose red colored zone was indicated positive for glucose/xylose isomerase.

Enzyme preparation

Streptomyces species were grown on peptone yeast extract broth containing 0.5M $MgSo_4.7H_2O$ and 0.1mM $CoCl_2.6H_2O$ with 1% inducer (Xylose). The pH of the medium 7.5 and the temperature was maintained at 40^{o}C for 5 days. After cultivation the cells were harvested by centrifugation at 10,000rpm for 30min. The wet biomass weighed and it was suspended in a 5ml of 50mM potassium phosphate buffer with 0.1mM EDTA. The cell suspension was applied on Ultrasonic disintegrator for cell disruption again centrifuged; the supernatant containing cell free extracts were used for enzyme activity [14].

Seliwanoff's reaction

Glucose isomerase production was confirmed by seliwanoff's test [15]. The reaction mixture containing 0.4ml of crude enzyme and 0.6ml of substrate (0.1M glucose) was made up the final volume with 2ml of deionized water, boiled at 75^{o}C for 30min. Finally 2ml of Seliwanoff's reagent was added and heated for 5min. The development of cherry red color indicates the occurrence of fructose due to the presence of glucose isomerase.

Thin Layer Chromatography for isomerization

Isomerization of glucose to fructose was detected by thin layer chromatography separation (Silica gel Merk) using a solvent system containing ethyl acetate, ethanol, water (4:5:1) ratio [16]. Conversion of fructose was identified by spraying 9ml of 2% resorcinol plus 1ml of Orthophosphoric acid. The plates were air dried and heated at 75^{o}C for 5min. The red color spot was indicated the isomerization reaction catalyzed by glucose isomerase.

Glucose isomerase assay

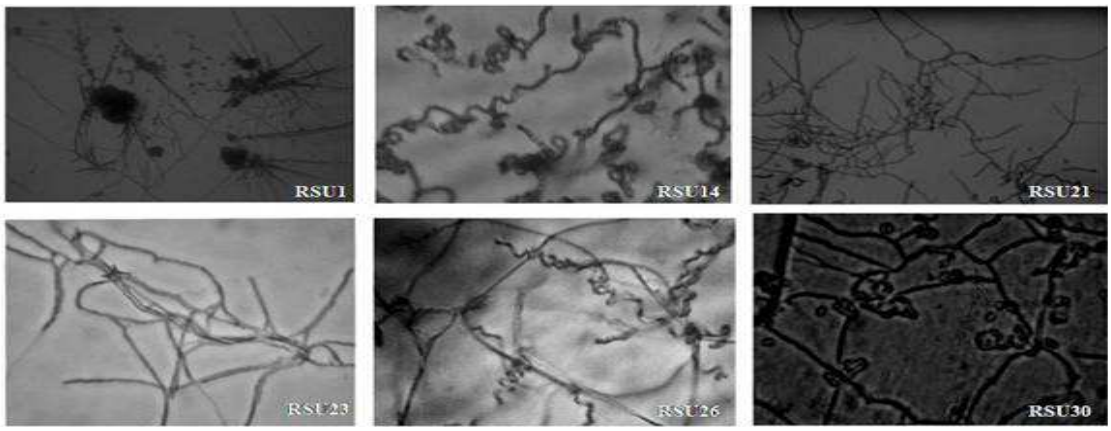
The production of glucose isomerase was detected the following method as described by Takasaki [16]. Enzyme reaction was performed using following chemical mixture consist of 0.4ml of 1M D-glucose,1ml of potassium phosphate buffer (pH.7.5), 0.2ml of 0.1M $MgSo_4.7\ H_2O$,0.2ml of 0.01M $CoCl_2.6H_2O$, 0.8ml of enzyme solution and the final volume was made up to 4 ml of deionized water and incubated for 30min at 65^{o}C, the reaction was stopped by adding 5ml of resorcinol reagent and then heated at 100^{o}C for 5min then store on ice for 10min, again centrifuged at 10000rpm for 15min. The absorbance and wavelength scan was read at 485nm using UV spectrophotometer with a standard fructose as a reference. 1 unit of the glucose isomerase activity was apparent as the total of the enzyme that produced 1µmol of D-fructose per min under the assay condition was employed.

RESULTS AND DISCUSSION

Isolation and identification

In this study the sediment sample were collected from muthupet mangrove forest. Starch casein agar medium was used for the isolation of *Streptomyces*. Totally 105 *Streptomyces* species were isolated from mangrove soil. From that predominantly occurring 30 *Streptomyces* species were selected for further research. Number of findings were reported, that the populations of *Streptmyces* are high in mangrove environment and produced novel compounds [17, 18]. Identification of *Streptomyces* was confirmed by morphological, cultural and biochemical characteristics. *Streptomyces* showed a notable array of macroscopic features such as pigmentation (yellow, violet, green, orange and black pigment), aerial, substrate mycelium and differences in colony color such as yellow, ash, white, brown, pink, gray etc. The SCA medium was mainly suitable one for isolation and identification of *Streptomyces*. Light microscopy of *Streptomyces* isolates was observed under oil immersion objective (Fig.1). Color of aerial mycelium is considered to be an important character for the grouping and identification of actinomycetes family particularly *Streptomyces* was studied Pridham and Treser [19]. Atalan [20] reported the appearance of smooth surface spores was characterized about 70 to 80% of the *Streptomyces*. The production of Citrate, Catalase, Urease, Nitrate and Carbohydrate fermentation (Sucrose and Glucose) has been considered for the identification of *Streptomyces*. Various bio chemical characteristics were used for identification of *Streptomyces* [21, 22]

Fig.1. Light Microscopy images of *Streptomyces* species



Screening of glucose isomerase

Among them morphologically distinct as well as dominantly found 30 *Streptomyces* species were selected for screening of glucose isomerase. *Streptomyces* species were produced intense zone and color on the production media, which indicates the presence of glucose isomerase producers. 17 numerous *Streptomyces* species was exhibited positive results. From that 6 species exhibited higher zone of clearance. The selected superior performing strains were analyzed further screening experiments. For the Fructose estimation, the development of cherry red color was observed on Seliwanoff's reaction. The screening of glucose isomerase (conversion of D-glucose to D-fructose) was identified by thin layer chromatography (Table.1). The isomerization of glucose to fructose was detected by the formation of red color spot on the TLC plate (Fig.2). This method was highly suitable and convenient one for glucose isomerase separation. Based on the higher zone of clearance, Seliwanoff's test for fructose determination and thin layer chromatography; potentially 6 *Streptomyces* species exhibited better result for

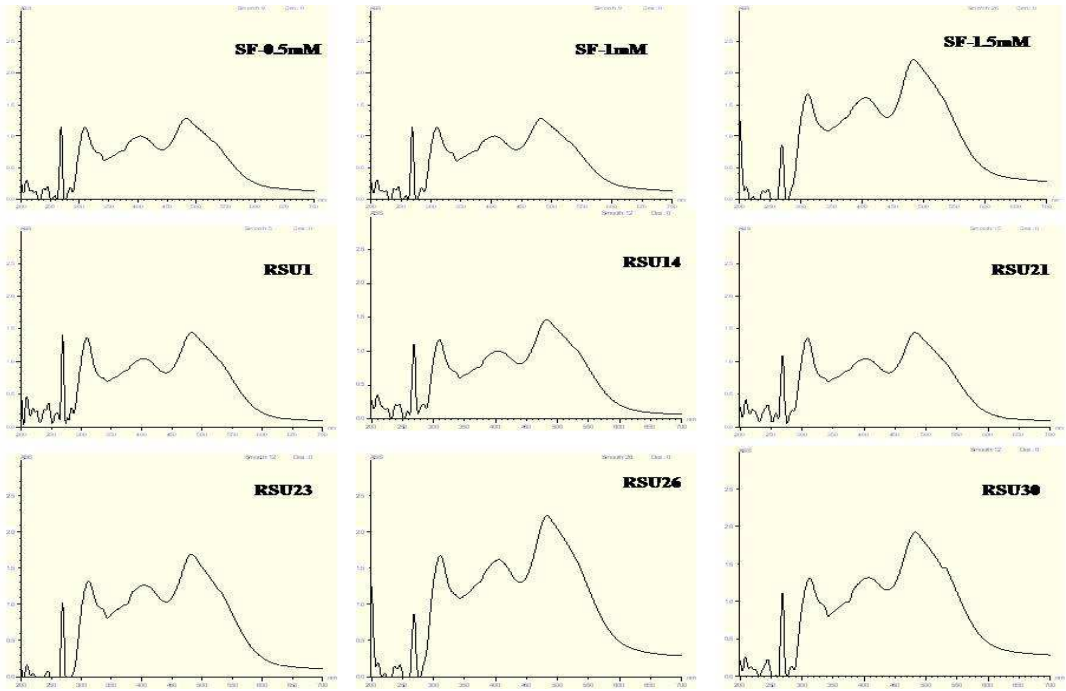
glucose isomerase production. Al Tai [23] reported some properties of glucose isomerase from *Streptomyces sp*. strain C7 exhibited glucose isomerase activity and identified the conversion of glucose to fructose by TLC.

Table.1. Glucose isomerase screening by Thin layer chromatography separation

Streptomyces isolates	Cell Biomass (mg)	Isomerization of glucose to fructose	TLC	R_f Value
RSU1	1150	+	+	0.7
RSU14	900	+	+	0.65
RSU21	850	+	+	0.6
RSU23	1005	+	+	0.7
RSU26	991	+	+	0.5
RSU30	890	+	+	0.55

Fig.2. Thin Layer Chromatography separation for Fructose

Fig.3. Glucose to fructose isomerization at 485nm Wavelength Scan



SF-Standard Fructose; RSU- Streptomyces isolates

Glucose isomerase Assay

The selected *Streptomyces* species were exhibited enzyme activity and correlated with standard fructose absorbance and wavelength scan was measured at 485nm using UV Spectrophotometer (Fig.3). The absorbance readings were predictable for glucose isomerase activity. The isomerization of glucose to fructose was highly observed in *Streptomyces* sp. RSU26; although *Streptomyces* sp. RSU14, RSU23 and RSU30 was moderately convert the glucose to fructose, lowest level of fructose conversion was observed in RSU1and RSU21. Based on the isomerization reaction the *Streptomyces* sp. RSU26 exhibits potent glucose isomerase activity among the different isolates. Hence, it is suitable one for the production of fructose.

CONCLUSION

In this study, isolated mangrove *Streptomyces* species has been potential to produced glucose isomerase enzyme. Glucose to fructose isomerization was catalyzed by cell free extracts of various *Streptomyces* species. A different intracellular glucose isomerase screening experiment was performed; even though plate assay and Seliwanoff's test provide good result for screening experiments but TLC is the most suitable and very quick method for glucose isomerase screening. Enzyme assay was proved, the isolated *Streptomyces* species exhibited intracellular glucose isomerase activity. Significantly *Streptomyces* sp. RSU26 was exceedingly performed for glucose isomerase production.

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